ABSTRACTS OF KEYNOTE LECTURES (KL), INVITED COMMUNICATIONS (IC), ORAL COMMUNICATIONS (OC), AND POSTERS (P).

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Session 1: Opening/From History to Genetics

KL1. Atopic Dermatitis and Dermatological Doctrines : A Historical Approach

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Atopic symptoms have been identified since Antiquity but there is still a lack of consensus about an accurate definition of the dermatological component of atopy. In this lecture we will propose that the definition and description of "atopic dermatitis" relies on the dominant medical doctrine. Before 1800, Hippocratic humoralism dominated the medical thought. Skin was considered as an excretory organ and oozing was perceived as a beneficial event. A therapeutic intervention leading to the termination of oozing could have deleterious consequences. Nevertheless, a disorder characterized by an itchy, predominantly cephalic rash in infants was readily identified, and described under various headings. Around 1800, Willan, Bateman and their followers started to look at the elementary lesions of skin disorders and proposed to classify dermatoses accordingly. This clinical approach was successful for diseases such as psoriasis, which could be rather well defined by squamous lesions. But a disorder where one can find papules, vesicles, pustules was not easily denominated. At the end of the nineteenth century, a wider attention for the symptom pruritus and for whole patient's clinical presentation led to the description of "diathetic prurigo". At the same time, pediatricians favored a digestive approach of infantile eruptions and the etiology of infantile eczema was looked for in dietary considerations. Shortly after the discovery of anaphylaxis and allergy, the positivity of skin and blood tests for proteins was discovered in children with eczema. It was thus felt that this was an important pathophysiological clue. In 1933, Wise and Sulzberger named atopic dermatitis and proposed the first criteria, including clinical data and the positivity of allergy tests. Confusion recently arouse when it became obvious that many patients with so-called atopic dermatitis do not have allergy stigmata.

In 2005, there are at least three doctrines allowing an approach of atopic dermatitis' definition:

1 – according to the allergological doctrine, AD is the cutaneous component of the atopic disease, an immunological disorder with hyper IgE and other well established immune abnormalities. Inside this doctrine, variants put the stress on milk, on other foods, on gut flora, . . .
2 – according to the dermatological doctrine, AD is an eczema, predominating in young children, with a genetic predisposition and characteristic clinical features, well described in 1980 by J Hanifin and G Rajka. The inflammatory symptoms are characteristically associated with epidermal abnormalities.
3 – according to the public health doctrine, AD is a chronic itch starting in infancy.

Therapeutic choices follow medical doctrines. Humoralist physicians did not want to interrupt the oozing. Allergologists favor hyposensitizations or avoidance regimens. Dermatologists treat the skin with anti-inflammatory topicals. They must face corticophobia, which is frequent in the lay public as well as among health professionals.

Scientific meetings such as the Rajka Symposias are needed to allow encounters of scientists defending different doctrines. In our opinion, theoretical discussions are interesting to understand the true meaning of medical approaches and to select the options the more likely to directly benefit patients.

KL2. Genetics and Epigenetics of Atopic Dermatitis

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Atopic Dermatitis is strongly familial and has a genetic as well as an environmental basis. Progress in understanding the genetics of AD is likely to accelerate in the post-genome era. Genetic Mapping studies have identified several chromosomal regions that contain genes predisposing to AD. Four of these regions correspond to known psoriasis loci, and one region in addition shows genetic linkage to psoriasis susceptibility. Detailed mapping of these loci is being carried out in several centres. Our group, in addition, has been carrying out gene expression analyses of keratinocytes during differentiation and the response to inflammatory stimuli. The results from these studies reveal much that is new about keratinocyte biology, as well as indicating key candidate genes for genetic mapping and association.

IC1. World Wide Variation of Risk Factors in Infants with Atopic Dermatitis

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Atopic dermatitis (AD) is a chronically relapsing, inflammatory skin disease with an early onset during infancy and a high impact on quality of life and socio-economic burden. Development of allergic diseases may be determined by some key factors in very early life. In some children there is also a progression from food allergy and atopic dermatitis to asthma. Early sensitisation to allergens is thought to be a key factor in the progression of the disease, from food allergy and atopic dermatitis to asthma as well as to persistent AD in later life. However, good epidemiological data about risk factors from different countries are missing. In our study children aged 1–2 years with active dermatitis and a family history of allergy were investigated in 12 countries (Australia, Austria, Belgium, Czech Republic, Germany, Spain, France, UK, Italy, The Netherlands, Poland, South Africa). Data were obtained on 2184 AD children with a mean age of 18 months; 57% were boys. The mean SCORAD was 33; 68% of the children had moderate-severe dermatitis (SCORAD $>$ 25). Two-thirds of the children were atopic (at least one specific IgE, or total IgE $>$ 30kU/l) with high variations among the countries. It can be clearly demonstrated that country specific exposures are significantly associated with early sensitisations. These findings are important with respect to prevention measures. In contrast to the exposure to cat only the exposure to dog was negatively associated with the sensitisation rate in AD infants and the exposure to cat and dog is associated with a lower sensitisation risk against cat allergen. Exposure to cat and/or dog did not increase the severity of AD in this age group. These findings might be important for guiding prevention measures in families with AD children. Additionally, the study gave new data about vaccination and AD. Common vaccinations in early childhood are mostly not positively associated with an increased risk of allergic sensitization or with more severe AD. In general, parents of children predisposed for atopy should be encouraged to provide them effective vaccination against potentially harmful diseases.

OC1. Microarray Analysis of Atopic Skin Lesions

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Background: Pathogenesis of atopic dermatitis has been attributed primarily to IgE elevation, and the disease has been classified as an atopic disease similar to allergic rhinitis or bronchial asthma. However, there have been few reports about the skin-specific abnormality on atopic dermatitis. Present study was designed to scoop gene expression which are specific to active atopic skin lesions.
Patients/Methods: We analyzed 23,000 genes in 17 atopic lesions and 4 normal controls using Affymetrix oligonucleotide arrays.
Results: Four of the 10 genes with the highest change in expression, S100A8 (32 up-regulated) and S100A7 (6.0 up-regulated), and loricrin (6.5 down-regulated) and filaggrin (5.1 down-regulated), were epidermal differentiation genes located in 1q21, a locus previously reported to have a genetic linkage with atopic dermatitis. Keratin 6 (locus12q13) and keratin 16 (locus17q12-q21) were up-regulated, whereas keratin 5, 14, 1, and 10 were not. Furthermore, the elafin (20q12-q13) and human β defensin 2 (8p23.1-p22) genes, both epithelial host defense proteins (6, 7), were not over-expressed in atopic skin lesions.
Conclusions: Our results, showing down-regulation of the cornified envelope genes and up-regulation of the alternative keratinization pathway, are the first to suggest abnormal epidermal differentiation and defective defenses as key abnormalities in atopic dermatitis.

OC2. Gene-Expression Profiling of Lesional and Atopy Patched Tested Skin in Patients with Atopic Dermatitis using cDNA microarrays

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We have used human cDNA microarrays to advance the understanding of Atopic Dermatitis (AD). The work presented here provides a detailed molecular picture of the programmed responses of the human genome to the pathological condition of AD. In addition we have performed immuno- histochemistry (IHC) studies to assess the generality of the results generated by the microarrays.

Skin biopsy samples were taken from two groups of individuals: AD patients sensitized to the opportunistic yeast Malassezia sympodialis and non-atopic healthy individuals (controls). Three skin biopsies were taken from each individual, i.e. lesional skin from AD patients (or healthy skin from control individuals), and M. sympodialis and PBS patch-tested skin. The cDNA microarrays used were produced at the Stanford functional genomics facility (http://www.microarray.org/sfgf/jsp/home.jsp), and contained $\sim$ 42.000 cDNA. Data were analyzed by different methods; both unsupervised (hierarchal clustering) and supervised methods (using SAM, Significan Analysis of Microarray and gene-lists).

We here present a picture of approximately 1,500 genes differently expressed between healthy control individuals and lesional skin from AD patients (who often showed the same expression pattern as M. sympodialis tested skin). A selection of these genes is described in more details in the context of their biological function and possible role in the pathogenesis of AD.

(I). Immune response; Increased expression levels were detected in the lesional and M. sympodialis tested skin of genes encoding the interleukin-4 receptor (IL-4R), interleukin-6 receptor (IL-6R) and the IgE receptor, gamma subunit (FCER1G) and genes encoding chemokines such as CCL2, CXCL1, CXCL3, CXCL4 and CXCL9. We observed a four-fold difference between involved and uninvolved skin of AD patients in the expression levels of SOCS-3. This result has been confirmed with IHC studies and further investigations of the SOCS-3 gene are ongoing.

(II). Barrier function; We identified a number of genes with lower expression levels in lesional skin compared to uninvolved skin of AD patients and control individuals. This included genes encoding known components of epithelial cell-to-cell junctions and enzymes involved in lipid metabolism.

(III). Defense mechanisms of the skin; It has previously been reported that antimicrobial peptides are produced at lower levels in the skin of AD patients compared with psoriasis. We identified a gene involved in biosynthesis of antimicrobial peptides (LANCL1) with decreased expression levels in lesional skin as compared to uninvolved skin from AD patients and healthy controls. Other genes expressed at a lower level in lesions were mucin 1 (MUC1; a glycoprotein with a role in protecting many epithelial surfaces) and secretory leucoprotease inhibitor (SLIP; with antibacterial, antiviral, and anti-inflammatory functions).

(IV). Signaling pathways; Genes encoding members of the epidermal growth factor receptor (EGFR) protein family showed decreased expression levels in lesional skin as compared to non-involved skin. The EGFR signaling pathway plays a key role in the regulation of cell proliferation, survival and differentiation.

In summary, we here report that lesional skin and induced eczema (i.e. M. sympodialis tested skin) share very similar gene-expression patterns. This initial study provides information about potential new candidate genes possibly involved in the pathology of atopic dermatitis, which could help explain abnormalities in AD skin.

OC3. Positional Cloning of a Susceptibility Gene for Childhood Eczema from Chromosome 1q21

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Eczema (atopic dermatitis, AD) is strongly familial and has a genetic basis. In order to identify genes predisposing to AD we have previously carried out a whole genome screen and have identified regions of linkage on chromosomes 1q21, 17q25 and 20p. The chromosome 1q21 locus overlies the epidermal differentiation complex (EDC). The genes of the EDC are expressed late during maturation of epidermal cells. A systematic search identified 189 single nucleotide polymorphisms (SNPs) in 54 genes from the EDC, including 35 coding for amino-acid changes. One hundred and eleven of these were typed in a panel of 150 nuclear families recruited through a child with severe eczema from clinics at Great Ormond Street Hospital (The ECZ1 panel). Forty-one SNPs showing potential association with AD by transmission/disequilibrium tests ($P < 0.01$) were carried through for typing on an additional panel of 278 families containing 470 sibling pairs (the MRC-E panel). One SNP showed strong evidence for replication of association to AD and to asthma (T60:NT30, $P = 0.0002$ and T53:NT14, $P = 0.000002$ respectively in the MRC-E panel). It and other associated SNPs were carried on a susceptibility haplotype that was highly significantly associated with eczema (T95: NT50, $P = 0.0002$ in the combined panels). The haplotype contains two genes that are over-expressed in skin biopsies of eczematous lesions. These have a potential role in modifying the response of the skin to infections.

OC4. The Links Between Gut Worms, Malaria, and Atopic Dermatitis: A Study in Rural Vietnam

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Background: Atopic dermatitis is becoming increasingly frequent in urban centres of developing nations. Developing countries such as Vietnam therefore appear ideal locations for the study of potential aetiological factors, with stark lifestyle differences between urban and rural areas. Various epidemiological studies have suggested that endoparasite exposure (e.g. to hookworm) may be an important factor for the urban/rural gradient of allergic disease. It is, however, possible that some of this effect is due to confounding or reverse causality, ie that atopics have an immune system that reduces parasite burden. Only an intervention study will be able to differentiate between causality, confounding and reverse causality.

Study design: Among 1824 primary and secondary schoolchildren in rural Vietnam, we are therefore conducting 1) a cross-sectional baseline survey to assess gut parasite and atopic dermatitis and asthma prevalences, followed by 2) a randomised, double blind, placebo-controlled trial of three-monthly gut parasite eradication to study the effect of loss of gut parasite infection on skin prick test positivity and clinical expression of atopic dermatitis and asthma.

Main outcome measures: At baseline, we use the International Study of Asthma and Allergies in Childhood questionnaire to gather information on allergic disease symptoms and lifestyle factors. All participants are also physically examined for atopic dermatitis, using the UK diagnostic criteria, and skin prick tested to Dermatophagoides pteronyssinus, Dermatophagoides farinae, and American cockroach. Gut worm infection is diagnosed qualitatively and quantitatively on stool sampling, using the McMaster method. Malaria status is determined with thin and thick blood films. In addition, a randomly selected subgroup of 200 secondary schoolchildren has donated venous blood for cytokine profiles, including IL-4, IL-5, IL-10, TNF-alpha, and IFN-gamma.

Following the initial baseline survey, all participants are randomised to either Mebendazole or placebo treatment, stratified by school, and given four times every three months. All baseline measurements are to be repeated at 8 weeks and 12 months into the study to examine whether phased gut parasite eradication promotes an increase in the clinical expression of atopic dermatitis and asthma and skin prick test positivity, and whether this also results in measurable changes in the host cytokine profile to explain pre and post parasite eradication differences in allergic disease prevalence.

P1. Epidemiological Characteristics of Atopic Dermatitis in India

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Atopic dermatitis (AD) is a common inflammatory skin disease, encountered in the pediatric age group. The prevalence of AD appears to have increased over the past three decades in western countries. Most of the epidemiological studies on AD are based on western population, with little published information in Asians and people from developing countries. In our earlier reports, we have studied the clinical profile of AD including major and minor criteria. We retrospectively analysed the pediatric dermatology clinic records to study epidemiological characteristics of the disease over the last 15 years. Diagnosis of AD was based on diagnostic criteria of Hanifin and Rajka. Based on body surface area involvement, disease was considered to be mild (<20%), moderate (20–50%) and severe (>50% body area involvement).

Of the total 271,839 new patients seen during this period in the Dermatology outpatient department, 1542 children had AD making an incidence of 0.56%. Out of 3521 total number of new patients seen in the pediatric dermatology clinic during the same period, AD comprised 43.8% of the pediatric dermatoses. Of the 1542 patients, 435 (28.2%) were infants (up to 1 year), of whom 295 (67.8%) were boys and 140 (32.2%) were girls with a male:female ratio of 2.07:1. Mean age at onset of the disease was 3.9 months and mean duration of disease was 3.1 months. 96 (22.0%) infants were from a rural background and 339 (78%) lived in urban area. A personal and family history of atopy was noted in 4 (0.91%) and 168 (38.6%) infants respectively. No patient had any suspected drug or food allergy.

Of the 1089 children with AD, 684 (62.8%) were boys and 405 (37.2%) were girls, with male: female ratio of 1.3:1. Mean age of onset and mean duration of disease was 3.6 years and 1.2 years respectively. 284 (26.0%) patients were from a rural background and 805 (73.9%) were from an urban area. Of these children 171 (15.7%) had a personal history of atopy, 467 (42.9%) had a family history of atopy and 85 (7.8%) had both personal and family history of atopy. Of the total 1542 patients, 1390 (90.1%) had mild disease, 121 (7.8%) had moderate and only 31 (3.2%) children had severe disease. Atopic dermatitis therefore appears to be a common problem with significant morbidity in developed nations but relatively of low prevalence and less severity in developing world. However, prevalence of AD in developing countries may increase as traditional lifestyle are eroded by increasing adaptation to the living patterns exhibited by industrialized societies.

P2. Worldwide Changes in the Prevalence if Eczema Symptoms

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Objective: We sought to determine if the prevalence of atopic eczema symptoms have increased worldwide.

Methods: We studied children (n = 298,080) aged 13–14 years in 104 centres from 55 countries around the world, and children aged 6–7 years (n = 185,891) from 64 centres in 36 countries who participated in successive cross sectional prevalence surveys. On average, the surveys were conducted 7 years apart in the same study centres using validated and translated questionnaires (Phase 1 and III of the International Study of Asthma and Allergies in Childhood). Children with a positive response to an itchy relapsing skin rash in the last 12 months that had affected the skin creases were considered to have eczema, and those with symptoms resulting in sleep disturbance for 1 or more nights per week were considered to have severe eczema.

Results: Changes in prevalence per year (weighted by the standard error and adjusting for clustering effects) over the 7 year period were generally small. Many of the centres previously showing the highest prevalences such as UK and New Zealand now show a levelling off or decrease in prevalence. Similar patterns are seen for those with severe eczema, suggesting that differential reporting of mild disease is unlikely to account for the observed changes. The pattern elsewhere is mixed, with many formally low prevalence countries experiencing substantial increases in eczema symptoms.

Interpretation: Our results suggest that the epidemic of eczema may be levelling off or even reducing in some countries with previously high prevalence rates. The study has also highlighted other areas of the world where an increase in eczema might become the focus of primary and secondary prevention programmes. The changing prevalence of eczema symptoms needs to be confirmed in other studies with objective disease markers in order to exclude changes in the use of the language used to describe eczema symptoms over time. The fact that measurable changes in symptoms occurred over such a short time suggest that environmental factors are important in determining eczema expression, and that a threshold effect may be operating whereby a number of susceptibles develop disease until a plateau of saturation occurs.

Session 2: Maturation of the Immune System

KL3. Perinatal Maturation of the Immune System: Implications for the Aetiology and Pathogenesis of Atopic Dermatitis and Related Diseases

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During fetal life, the developing immune system is shielded from the antigenically hostile external environment, and the major challenge to homeostasis is regulation of potential fetomaternal immunological interactions which pose a threat to placental integrity. The mechanisms selected via evolution for this purpose, centering upon attenuation of fetal adaptive immune capacity and attendant Th2 polarisation, carry over into the immediate postnatal period, and contribute to risk for a number of immunoinflammatory and infectious diseases. Atopy represents an archetypal example of a disease in which risk for disease development is closely related to immune status at or around the time of birth. Accumulating evidence suggests that the kinetics of postnatal maturation of immune function is extremely heterogeneous with the human population, in particular maturation of Th1 function which is differentially attenuated during fetal life. Moreover, genetic risk for atopy and associated diseases appears inversely related to the kinetics of this maturation process, high risk being strongly associated with delayed kinetics. A series of mechanisms contribute to this risk, including those directly associated with regulation of expression of individual Th1 genes such as IFNγ, and those associated with the efficiency of recognition and transduction of the microbial derived signals from the postnatal environment (notably genetic variations in TLR function) which drive Th1 maturation. Additional developmental variations recently documented within the Eosinophil compartment also contribute to this process. Accumulating evidence also suggests that the developmental deficiency in Th1 function in high risk children is in many cases transient, and is followed in a significant number of cases by hyperresponsiveness in both the Th1 and Th2 compartments. This finding has important theoretical implications in relation to drug design, as it implies that the same Th1 cytokines which provide negative feedback to protect against initial allergic sensitisation have the potential to contribute to atopy pathogenesis once the disease is established. This concept is already established in the adult atopic dermatitis literature, and data will be presented on its relevance to the pathogenesis of allergic respiratory diseases in children.

KL4. Gut Microbiota and Atopic Dermatitis

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Atopic dermatitis is considered to result from an interplay between susceptibility genes, impaired barrier functions of the skin and the gut, aberrant gut microbiota, immunological dysregulation, together with bacterial and viral infections and other environmental factors. The hygiene hypothesis conceives the increase in the prevalence of atopic disease to be related to reduced exposure to microbes at an early age. The earliest and most massive source of microbial exposure is associated with the establishment of the gut microbiota.

The importance of the immunoregulatory potential of gut microbiota is emphasized in the recent demonstration of cross-talk between the innate and the adaptive immune system. Gut microbiota provide maturational signals for the gut-associated lymphoid tissue, particularly for the IgA plasma cells, conferring the first line of host immunological defence. Abundant IgA antibody production at mucosal surfaces contributes to the intestinal barrier function by binding to and excluding antigens. Maturation of dendritic cells carrying commensals and subsequent secretion of cytokines and chemokines then influence the polarization of T helper cells and thereby the adaptive immune responses, ensuring a local IgA response. This type of immune response has been suggested to prevent commensals from breaching the gut mucosal barrier, while pathogenic bacteria preferably destroy it.

Probiotics are live microbial food supplements or components of bacteria which have been demonstrated to have beneficial effects on human health. The probiotic effects in atopic eczema have been attributed to restoration to normal of increased intestinal permeability and unbalanced gut microecology, improvement of the intestine's immunological barrier functions, including the production of anti-inflammatory IL-10 and TGF-beta, and reduced generation of proinflammatory cytokines characteristic of local and systemic allergic inflammation.

Recent clinical studies have demonstrated a significant improvement in the clinical course of atopic eczema in infants and children given probiotic-supplemented elimination diet, and in parallel, markers of intestinal and systemic allergic inflammation decreased significantly. Moreover, probiotics administered pre- and postnatally for 6 months to children at high risk of atopic diseases, succeeded to reduce significantly the prevalence of atopic eczema as compared with that in infants receiving placebo.

Diet is always a combination of many foods and nutrients – their interactions may be more crucial than is currently understood. Therefore, rigorous scientific effort is required to elucidate how the dietary context interacts with probiotic supplementation on the complex cascade of immunological mechanisms in atopic disease.

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OC5. Numerical and Functional Abnormality of Circulating Plasmacytoid DC in Atopic Dermatitis

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Atopic dermatitis (AD) is a pruritic, chronically relapsing skin disease in which Th2 cells play a crucial role in cutaneous and extracutaneous immune reactions. In humans, CD11c + CD123- myeloid dendritic cells (mDC) and CD11c-CD123 + plasmacytoid DC (pDC) orchestrate the decision-making process in innate and acquired immunity. Since the number and function of these blood DC subsets reportedly reflect the host immune status, we studied the involvement of the DC subsets in the pathogenesis of AD. Patients with AD had an increased pDC number in the peripheral blood with correlation to AD-relevant immune parameters including the total serum IgE level and the ratio of interferon-gamma (IFN-g)-producing blood cells/interleukin-4 (IL-4)-producing blood cells as well as the disease severity estimated by SCORAD, suggesting pDC play a critical role for AD pathogenesis. In skin lesions, pDC infiltration was in close association with blood vessels expressing peripheral neural addressins. In vitro allogeneic stimulation of naïve CD4 + T cells with normal DC showed that the ability of mDC for IFN-g-producing cell induction was superior to pDC. On the other hand, atopic DC promoted IFN-g-producing cell differentiation comparable to normal mDC. Although pDC in both AD and normal subjects promoted differentiation of naïve CD4 + T cells into CD4 + CD25 + CTLA-4 + Foxp3 + T cells with regulatory function to T cells which represent peripheral regulatory T cells (Treg), these cells induced by atopic, but not normal, pDC had the ability to produce high amounts of IFN-g and IL-5. Taken together, atopic pDC have numerical and functional abnormality compared with normal pDC. In normal subjects pDC may migrate into inflammatory sites and promote Th2- and regulatory T cell-differentiation for dampening inflammatory responses. However, atopic pDC may promote Th1or Treg with high potential for IFN-g and IL-5 production, and thus protracting Th1-type and/or eosinophilic inflammation via Th1 cell activation and eosinophil trafficking on site in AD.

OC6. The Severity of Atopic Dermatitis Correlates to Thymic Function

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We have evaluated thymic function in relation to atopic dermitatis. Fluctuations in thymic output in patients with atopic dermatitis were measured by T-cell receptor excision circles (TREC) in peripheral blood CD4 + and CD8 + T-cells over a time period. TREC is an episomal DNA circle produced during T-cell receptor rearrangement and is used as a marker for recent thymic emigrants.

A group of 10 patients with atopic dermatitis were observed over a period of six months. Each month the patients were clinically scored with SCORAD and blood samples were obtained. To investigate the variance in TREC content in healthy individuals, a group of seven healthy persons were observed over a period of four months.

Over time, normal healthy volunteers showed very little change in their TREC, supporting that their thymus is working at a constant level. This is in contrast to atopic dermatitis where, over a time period of 6 months, we observed a large variation in the amount of TREC in lymphocytes. This variation was observed in both the CD4 + and CD8 + T-cell subpopulation. In comparison to healthy controls this variation was significant ($P = 0{,}022$ and $P = 0{,}008$ for CD4 and CD8 respectively).

Examining the individual components of SCORAD, TREC in both the CD4 + and CD8 + T-cell subpopulation was reversely correlated both with intensity (CD4 + $r = -0{,}27$, $p = 0{,}045$ and CD8 + $r = -0{,}35$, $P = 0{,}010$) and extent (CD4 + $r = -0{,}34$, $p = 0{,}008$ and CD8 + $r = -0{,}40$, $P = 0{,}003$), whereas the subjective symptoms of itching and sleep loss did not show any correlation.

This investigation supports a connection between thymus function and AD disease, and suggests that atopic dermatitis patients seems to have an increased emission of recent thymic emigrants during disease flare-up, supporting our hypothesis that atopic dermatitis is associated with altered thymic emission.

OC7. Probiotics Provide Clinical Benefit in Moderate and Severe Atopic Dermatitis: A Randomised Controlled Trial

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Introduction: The incidence of atopic dermatitis (AD) has increased dramatically in recent years. The resulting greater morbidity has highlighted the need for novel strategies to reduce the burden of disease. Probiotic supplements may provide protection from allergic disease, and improve the symptoms of infants with mild AD, but there is little data on the effects of probiotics in young children with more severe disease. The rationale for use of probiotic bacterial products as a therapeutic option in AD has been based on the well-recognised effects of bacteria on cellular immune responses.

Objective: To investigate the effects of probiotics on moderate or severe atopic dermatitis (modified SCORAD greater than 25) in young children (6-18 months of age) in a randomised, placebo controlled, double blind trial. Both clinical and immunological outcomes were assessed.

Methods: Fifty six children aged 6-18 months with moderate or severe atopic dermatitis were randomised to supplementation with probiotic (3x109 *L fermentum* PCC™, [VRI BioMedical]), or equivalent volume of placebo, twice daily for eight weeks. A final assessment at sixteen weeks was performed (8 weeks after end of intervention). Severity and extent of AD were measured by the SCORAD index at week 0, week 8 and week 16. Peripheral blood mononuclear cells were stimulated with heat killed *L fermentum* (HKLB). Cytokines (IL-5, IL13, IL 10, IFNgamma, TNFalpha) were measured in the supernatants.

Results: The reduction in SCORAD index over time was significant in the probiotic group ($p = 0.03$) but not the placebo group. Significantly more children receiving probiotics ($n = 24$, 92%) had a SCORAD index that was better than baseline at week 16 compared with the placebo group ($n = 17$, 63%) ($p = 0.01$). At the completion of the study more children in the probiotic group had mild AD ($n = 14$, 54%) compared to placebo ($n = 8$, 30%). TNFalpha responses to HKLB were significantly higher in the probiotic group at week 8 ($p = 0.022$) compared to placebo. Of note no other cytokine responses were significantly affected.

Conclusion: Supplementation with probiotic *Lactobacillus fermentum* PCC™ is beneficial in improving the extent and severity of atopic dermatitis in young children with moderate or severe disease, with persisting clinical benefits 2 months after completion of supplementation. This study demonstrates that exposure to *L fermentum* PCC™ in the gut has an effect on systemic TNFalpha responses. The effects of bacterial products on underlying immune responses require further investigation.

OC8. Involvement of Innate Conditioning of Antigen Presenting Cells by *Staphylococcus Aureus* Toxin B in Immune Response of Atopic Dermatitis Patients

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Atopic dermatitis (AD) is dominated by the development of specific T_H2 responses after exposure of the skin to environmental antigens. This immune deviation may reflect alterations in innate immune mechanisms. Recently, it has become clear that the development of T_H1 or T_H2 subsets is strongly influenced by factors produced by antigen presenting cells (APCs), thus representing a link between innate and adaptive immunity.

Toll-like receptors (TLRs) are members of pattern recognition receptors family on APCs that are activated by specific microbial components and some host molecules. They constitute the first line of defence against many pathogens and play a crucial role in innate immunity. Furthermore, TLRs were recently demonstrated to take part in the development of adaptive immune responses through activation of APCs.

We showed in a previous study that *Staphylococcal aureus* enterotoxin B (SEB) could induce activation of monocytes-derived dendritic cells (DCs) through toll like receptor 2 signalling and that SEB-pulsed DCs induced priming of naïve T cells and their commitment into T_H2.

To clarify whether APCs from AD patients could play a role in T_H2 cell polarization, we compared the expression of surface markers and cytokine production by monocytes of 30 AD patients (15 adults and 15 children) with those of 15 healthy controls, after stimulation with either LPS or SEB.

First, we observed that contrary to normal subjects atopic dermatitis patients were not able to further modulate TLR 2 and TLR 4 expression after *in vitro* exposition to SEB. Accordingly, cells seemed to be blocked in an activated stage with in addition no modulation of HLA-DR and CD54 expression. This feature was supported by secretion of large amounts of inflammatory cytokines (IL-6, IL-10, TNF-α) even in the absence of stimulation. Then, we observed that atopic monocytes did not produce bioactive IL-12 (IL-12p70) in response to LPS contrary to normal subjects but that secretion could persist in atopic children. These findings demonstrate that cytokine responses changed markedly between normal and atopic persons and provide further evidence of a close relationship between Th2 skewing and disease progression. Moreover, these data suggest a major role of innate immunity through activation of TLRs on skin APCs in pathogenesis of AD.

P3. Probiotics in the Management of Atopic Dermatitis

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Background: Recent studies suggest that oral bacteriotherapy with probiotics might be useful in the management of atopic dermatitis.

Objectives: The purpose of this investigation was to evaluate the efficacy and safety of treatment with probiotics in adult atopic dermatitis as well as that of childhood.

Methods: 60 patients with mild to moderate atopic dermatitis were recruited in the treatment of a mixture of four probiotic strains (Lactobacillus rhamnosus, L. plantarum, L. casei, Bifidobacterium lactis) twice daily for 8 weeks. The degree of pruritus was determined by using the 10-point visual analogue scale and the patients' subjective evaluations (ie, better, unchanged, or worse) were assessed every other week.

The clinical severity of the eczema was evaluated by using eczema area and severity index (EASI). As laboratory markers, total IgE, eosinophil cationic protein in serum and cytokine production (IL-2, IL-4, IL-10, IFN-gamma, TGF-beta) by peripheral blood mononuclear cells were measured.

Results: Of the 60 mild to moderate atopic dermatitis patients enrolled in the study, 3 patients dropped out due to diarrhea, abdominal pain or aggravation of atopic dermatitis and 7 patients were lost to follow-up. After treatment, 81% of the patients experienced improvement of the eczema and 65% of the patients experienced improvement of pruritus.

Conclusions: A mixture of four probiotic strains was beneficial in the management of atopic dermatitis. Thus, probiotic approach may offer a benefical direction in maintaining atopic dermatitis in a stable state as a supplemental therapy.

P4. The IgE-Bearing B-Cell Receptor Repertoire of Atopic Dermatitis Patients Shows Unbiased VH-Usage but Patient-Specific Clonal Expansions Regardless of Serum IgE Levels

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Previous studies have postulated a bias in the usage of IgE-associated VH-chains in atopic individuals favouring a preferential usage of the VH6 segment. We have analyzed CD19-sorted B-lymphocytes from several adult atopic eczema patients displaying very high serum IgE levels (>1000 IU/ml). These purified B-cells were screened in a quantitative Real-Time RT-PCR technique for their transcription of all eight VH-genes together with CH-primers of the e-, g1-4, and m-type, representative for the repertoire of IgE-, IgG-, and IgM-families. The VH-CHe amplifications were analyzed for their complementary determining region 3 (CDR3) spectra by a modified Immunoscope technique. In some patients, clonal expansions were further sequenced and compared to corresponding expansions of the IgM and IgG transcripts in order to screen for somatic mutations of the expanded clones and/or commonly selected clones. Serum IgE levels and antigen-specific IgE were determined using the IMMULITE system.

Our approach revealed a preferential usage of VH3b-, VH4-, and VH1-chains in IgE-producing B-cells regardless of the individual sensitization pattern. These chains were also preferentially found in IgG and IgM transcripts. Each patient, however, harbored specific clonal expansions which were identified by the CDR3-spectratyping and which were not shared between patients even in cases of highly similar specific IgE production. Extensive sequence analysis revealed unique CDR3-sequences showing somatic mutations. However, sporadic clonal sequences were shared between IgE- and IgG transcripts suggesting a common origin.

Atopic individuals with high IgE levels show a highly individual pattern of IgE-bearing B-cell expansions with no preferential VH-usage but particular CDR3-composition.

Session 3: Infection and Imunity

KL5. The Role of Infection in Atopic Dermatitis

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Infection plays an important role in atopic dermatitis (AD). Much research has focused on Staphylococcus aureus, which appears to mediate its proinflammatory effects at least in part via the production of superantigens. Epicutaneous application of superantigens induces eczema and enhances Th2 skin responses. Most AD patients make specific IgE antibodies directed against staphylococcal superantigens; and these IgE anti-superantigens correlate with skin disease severity. Superantigens also induce corticosteroid resistance, expand skin homing T cells and subvert T regulatory cell activity suggesting that several mechanisms exist by which superantigens increase AD severity.

Increased binding of S. aureus to skin is driven by underlying AD skin inflammation. This is clinically supported by studies demonstrating that treatment with topical corticosteroids or calcineurin inhibitors reduces S. aureus counts on atopic skin.In experimental animal models, S. aureus binding was significantly greater at skin sites with Th2-, as compared to Th1-, mediated skin inflammation due to IL-4 induced expression of fibronectin. AD skin has also been found to be deficient in antimicrobial peptides needed for host defense against bacteria, fungi and viruses. Thus, once S. aureus binds to AD skin, inadequate local host defense allows bacteria to colonize and grow. The lack of skin innate immune responses predisposes these patients to infection.

Patients with AD also have an increased propensity toward disseminated viral skin infections with herpes simplex or vaccinia virus (VV). Investigations into eczema vaccinatum have intensified due to recent interest in mass smallpox vaccinations since 10–20% of children have AD and these children carry a life-long susceptibility to eczema vaccinatum. Interestingly, we have found that skin explants from AD patients support significantly higher levels of VV replication as compared to normal or psoriasis skin. Conversely, VV stimulation induces higher levels of LL-37 gene and protein expression in psoriasis skin and normal skin as compared to AD skin. Stimulation of human keratinocytes with VV induces LL-37 expression. However, IL-4/IL-13 down-regulates the expression of LL-37 in VV-stimulated keratinocytes. Additionally, VV replication significantly increases in keratinocytes treated with IL-4/IL-13. Using antibodies to IL-4/IL-13, we have demonstrated that neutralization of Th2 cytokines in AD skin augments LL-37 expression and inhibits VV replication.

Since LL-37 kill VV, its reduction in AD may predispose AD patients to eczema vaccinatum. The mechanisms for these reduced antimicrobial properties in AD skin appear to be due to a combination of increased IL-4, IL-10 and IL-13. New strategies for controlling propensity for viral infection in AD may include downregulation of Th2 cytokine expression in AD or development of drugs with cathelicidin-like activities which can be used as anti-viral agents.

IC2. Inflammatory Reactions to *S. Aureus* in AD

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It has been well established that atopic dermatitis (AD) disease is complicated by the concurrent skin colonization with Staphylococcus aureus. Many, but not all, S. aureus strains carry the capacity to produce exotoxins with superantigenic properties able to activate T cells in an unspecific manner. In this context local application of superantigens triggers recruitment and activation of T cells in and to the dermis. More recently, staphylococcal superantigens have been shown to inhibit the suppressive function of regulatory T cells of AD patients. Furthermore, a number of patients suffering from severe AD respond to staphyloccocal exotoxines with a specific immune response which is associated with detectable specific T-cell responses and specific IgE levels in the circulation. Since AD improves also in patients colonized with non-toxigenic S. aureus strains upon antimicrobial treatment, other S. aureus derived factors must be involved in the pathogenesis of AD. As shown by us recently, a high percentage of these bacteria is able to produce α-toxin, a pore forming cytotoxin. A wide range of human cells, among them keratinocytes and lymphocytes, tend to lysis by α-toxin with greatly varying susceptibility. In keratinocytes, TNF-α is released subsequently to lysis. Sublytic concentrations of α-toxin have paradox stimulating effects on human T cells which respond very efficiently with proliferation and IFN-gamma secretion to α-toxin. Since enhanced staphylococcal colonization is obviously not due to defects in acquired immunity, recent research on impaired responses to Staph aureus in AD was focussed on the innate immune system: Some studies have reported that epidermal keratinocytes in AD are incapable to up-regulate the production of some antimicrobial peptides (HBD-2, HBD-3, LL24) and that the combination of IL-4 and IL-13 inhibits the gene expression of those peptides. These findings point to the fact that the lack of these peptides may indeed account for the susceptibility of AD patients to bacterial infections in the skin compartment. In addition, a single nucleotide polymorphism (SNP) for TLR-2 (TLR-2 R753Q in the intracellular portion of the receptor) has been described which is associated with S. aureus infections. Interestingly, heterozygous AD-patients with the mutant TLR-2 R753Q allele exhibited a unique phenotype characterized by severe eczema (median SCORAD score 56 pts ranging from 30 to 90.5 pts) and high IgE levels in cohort investigated recently by us. None of them had a SCORAD score <30 pts, whereas the median SCORAD score of the non-polymorphic AD group was 45 pts (15 to 89 pts; min–max values). Since cell wall constituents of staphylococcal aureus are known to directly interact with TLR2, this polymorphism may also be involved in the susceptibility of AD patients to staphylococcal infections of the skin.

OC9. Th2 Cytokines Down-Regulate Cathelicidin Expression and Increase Skin Susceptibility to Viral Infection in Atopic Dermatitis (AD) Patients

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Patients with atopic dermatitis (AD) are susceptible to the development of eczema herpeticum and eczema vaccinatum. The development of these potentially life-threatening and disfiguring viral infections in AD patients is not well understood. Therefore despite renewed interest in mass vacation against smallpox, the CDC currently recommends that AD patients should not be voluntarily vaccinated against smallpox even after they have outgrown AD. Previously our group demonstrated that the cathelicidin anti-microbial peptide family exhibits anti-viral activity against vaccinia virus (VV) and is deficient in AD skin. Current studies were carried out to better understand the relationship between VV replication and cathelicidin expression in AD versus normal skin. Viral replication and cathelicidin expression were evaluated in cultured keratinocytes as well as skin explants from non-lesional AD ($n=6$), psoriasis ($n=6$) or normal skin ($n=7$) using real-time RT-PCR and immunohistochemistry. VV-DNA directed RNA polymerase gene expression was measured as an indicator of viral replication. Stimulation of keratinocytes with VV induced cathelicidin expression in a time- and dose-dependent manner ($p<0.05$). Addition of VV to skin explants resulted in significantly ($p<0.05$) higher levels of viral replication in AD (4854 $\pm$ 2240ng VV-DNA directed RNA polymerase) as compared to normal (519 $\pm$ 26) and psoriasis (807 $\pm$ 242). Conversely, VV induced higher levels of cathelicidin gene ($p<0.05$) and protein ($p<0.05$) expression in skin from psoriasis patients and normal subjects as compared to AD patients. Treatment with IL-4 and IL-13 prior to infection down-regulated ($p<0.001$) the expression of cathelicidin in VV-stimulated keratinocytes. Additionally, viral replication significantly increased ($p<0.001$) from 12760 $\pm$ 1263ng to 21280 $\pm$ 4814 in keratinocytes treated with IL-4 and IL-13. Using antibodies to IL-4 and IL-13, we found that neutralization of Th2 cytokines in AD skin augmented LL-37 expression and inhibited viral replication. Th2 cytokines in AD skin may block cathelicidin expression. Since cathelicidins kill VV, its reduction may potentiate VV replication in AD skin and predispose AD patients to adverse reactions from smallpox vaccination. Investigations are in progress to determine whether a deficiency in LL-37 accounts for increased susceptibility of AD patients to eczema herpeticum.

OC10. Microanalysis of Anti-Microbial Peptide, beta-Defensin-2, in the Stratum Corneum from Atopic Dermatitis Patients

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We have previously reported the possibility that vulnerability to bacterial colonization in the skin of patients with AD is associated with reduced levels of a natural anti-microbial agent, sphingosine, which results from decreased levels of ceramides as a substrate and from diminished activities of its metabolic enzyme, acid ceramidase. On the other hand, antimicrobial peptides such as defensin and cathelicidin have recently been elucidated to play an important role in host defense and cutaneous innate immunity. Although beta-defensin-2 is reported to be down-regulated in the whole skin of AD compared with psoriasis, little is known about their role in the colonization of S. aureus in the stratum corneum from AD patients, because a precise evaluation of these peptides for the quantitation in the stratum corneum as a front anti-microbial barrier against S. aureus colonization has not been yet performed. In this study, we have developed microanalysis of beta-defensin-2 in the stratum corneum by combination of immunoprecipitation and Western blotting and compared beta-defensin-2 content (expressed as ng/micro-g keratin) between AD and healthy control. Microanalysis revealed that beta-defensin-2 in the stratum corneum significantly increases in the AD lesion and occurs at a similar level in the AD non-lesion compared with healthy control. The beta-defensin-2 in the lesional skin also increases in proportion to the severity of AD. The counting of bacterial colonies revealed that there is a higher population of S. aureus on the lesional and non-lesional skin surface of patients with AD compared with healthy controls. Comparison between the colony counting and beta-defensin-2 in the same skin sites demonstrated that there is a positive correlation ($r=0.460$, $p=0.021$, $n=25$) between these factors. Collectively, these findings suggest that beta-defensin-2 becomes inducible in response to bacteria, injury or inflammatory stimuli and is not associated with vulnerability to S. aureus colonization in the skin of patients with AD.

OC11. The Balance Between Langerhans Cells and Inflammatory Dendritic Epidermal Cells as a Regulator of Immunogenic and Tolerogenic Immune Responses in Atopic Eczema

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Two different dendritic cell subtypes are the key players in the epidermal skin lesions of atopic eczema (AE): Langerhans cells (LC), which are localized in the lesional and non-lesional skin and Inflammatory Dendritic Epidermal Cells (IDEC), which are recruited into the skin at the initiation of AE and only present at inflammatory epidermal sites. Considering the pathophysiology of AE, IDEC are regarded as the main amplifiers of immunogenic allergic inflammatory immune responses in the skin, while LC have been shown to be capable to some degree to induce immuno-tolerance in a constantly antigen exposed epidermal environment. Data supporting the idea of a critical balance between LC and IDEC in AE in this context are still lacking. In the last decade, calcineurin inhibitors such as tacrolimus (FK506) have been successfully introduced as an effective treatment of AE. Furthermore it has been described that tacrolimus treatment diminishes the number of IDEC cells in the skin and restores the physiological supremacy of LC, although the underlying immune mechanism leading to this phenomenon remains unknown.

We observed that treatment of differentiating DC from patients with AE with tacrolimus induces the production of transforming-growth factor (TGF)-beta2 and TGF-beta3 by DC, which triggers the generation of high numbers of LC in a TGF-beta-dependent manner. Further on tacrolimus modifies the expression co-stimulatory and co-inhibitory molecules involved in the induction of tolerogenic mechanisms such as B7-H1, B7-H2, B7-H3 molecules on the surface of LC while decreasing the stimulatory capacity of LC toward autologeous and allogeneic T-cells in T-cell proliferation assays.

Interestingly, the down-regulation of the stimulatory capacity of tacrolimus-treated LC corresponds with the induction of T-cells with a high IL-10 and TGF-beta producing capacity. Beside that we could show that the suppression of T-cell responses initiated by tacrolimus-treated LC is dependent on the presence of CD4 + CD25 + regulatory T-cell subsets within the T-cell fraction. These mechanisms are dependent on LC/T-cell -contact and revertible by the addition of IL-2 to the T-cell culture, which is known to inhibit suppressive functions of CD4 + CD25 + T-cells. From our data a picture emerges that the tacrolimus-promoted differentiation of LC from precursor cells recruited into the skin and DC present within the skin might skew the balance from immunogenic IDEC toward tolerogenic LC and lead to an overbalance of tolerogenic LC which are capable to induce functionally active regulatory T-cells. Moreover, the introduced principle of a critical interplay between IDEC and LC in AE, designating the outcome of either a tolerogenic or an immunogenic state in the skin opens a new way for therapeutic strategies. These might be aimed to target and facilitate the function of LC as potential natural silencers of allergic-inflammatory immune responses.

P5. Aberrant Blood Dendritic Cells in Atopic Dermatitis

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Dendritic cells (DC) are professional antigen presenting cells that play a pivotal role in the orchestration of Th cell responses. Myeloid (BDCA1) and plasmacytoid (BDCA4 and interferon-producing cells) DC have been described, that differ in their responsiveness to pathogenic compounds and their cytokine production profile. The function of these DC subsets in atopic disorders is not well studied. Therefore, we compared the frequency and cytokine profile of these subsets in peripheral blood from AD patients and healthy controls. No differences were found in the frequencies or the expression of the T cell stimulatory molecule HLA-DR and the T cell co-stimulatory molecules CD86 and CD83. Analyzing their cytokine producing capacity, we found no differences in the production of pro-inflammatory TNF-alpha or anti-inflammatory IL-10 in BDCA1 or BDCA4 cells. However, BDCA1 and BDCA4 DC from AD patients showed a selectively and strongly reduced capacity to produce the Th1-polarizing cytokine IL-12p70, and the anti-viral and Th1-polarizing cytokine IFN-alpha, respectively. Since reduced IL-12 or type I IFNs may promote Th2-biased responses, these data suggest that DC from AD patients have a role in the induction or maintenance of the Th2-associated allergic response. Moreover, reduced anti-viral IFN-alpha production by BDCA4 cells may be instrumental in the increased susceptibility to virus infection in AD.

Session 4: Epidermal Inflammation Including Neurogenic Inflammation and Pruritus

KL6. Chemokines in Atopic Dermatitis

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Atopic dermatitis is a chronic or chronically relapsing inflammatory skin disease with eczematous lesions demonstrating typical morphology and distribution, severe pruritus, elevated serum IgE, the presence of allergen-specific IgE, and peripheral blood eosinophilia. The prevalence of atopic dermatitis rapidly increased during the past decades and is currently ranging between 10–20% in children and 1–3% in adults. Histopathologically, the lesional skin of atopic dermatitis patients shows a dermal infiltrate consisting of mainly activated cutaneous lymphocyte associated antigen (CLA)$^+$ memory T cells (CD4 > CD8) and antigen-presenting cells (APC). Among the APC population, lesional skin shows increased numbers of Langerhans cells (LC), inflammatory dendritic epidermal cells (IDEC), as well as dermal dendritic cells which show markedly upregulated expression of Fc receptors for IgE on their cell surface. Moreover, dermal sites of atopic skin demonstrate extensive deposition of eosinophil-derived proteins or more rarely intact eosinophils. Exposure to allergens, e.g. house dust mite antigens, or microbial products plays an important role in the initiation and maintenance of atopic skin inflammation. In early phases of the disease, memory T cells with a T_H2 phenotype infiltrate the atopic skin; however, chronic lichenified atopic dermatitis lesions are characterized by the dominance of skin-infiltrating T_H1 cells.

In the past decade, numerous studies identified chemokines associated with atopic dermatitis. These chemokines include CCL1, CCL2, CCL3, CCL4, CCL5, CCL11, CCL13, CCL17, CCL18, CCL20, CCL22, CCL26, CCL27, and CX_3CL1. Notably, serum levels of CCL11, CCL17, CCL18, CCL22, CCL26, CCL27 and CX_3CL1 directly correlated with disease severity suggesting an important role in the immunopathogenesis of atopic dermatitis. Among these chemokines, CCL1, CCL17, CCL18, CCL22 and CCL27 are likely candidates to critically regulate the recruitment of memory T cells to sites of atopic skin inflammation. Here an overview is provided of the role of chemokines in the complex immunopathogenesis of atopic dermatitis, highlighting potential areas for therapeutic intervention.

IC3. Is Atopic Dermatitis a Neurogenic Inflammatory Disease?

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Newer studies try to point out the close connection between epidermal nerve fibers and brain, together with new developments in brain research and studies with neuropeptides in skin diseases which are mainly involved with epidermal nerve fibers (Undem et al 2000). In the past years it becomes more and more evident that atopy-relevant effector cells, such as mast cells and Langerhans cells form a close anatomical relationship with nerve fibers staining positive for a number of neuroactive substances, for instance substance P, vasoacitve peptide or Neutrophine Growth Factor NGF (Gieler et al 2002). Regarding this close anatomical relationship of nerve terminals and effector cells in atopic eczema, it seems possible that stress-induced stimulation of nerve fibers induces secretion of neuroactive substances. There are a growing number of studies indicating that atopic eczema patients show disturbances in neuroimmunological pathways so that some authors stated that psychological stress may be conceptualized as a social pollutant that, when 'breathed' into the body, may disrupt biological systems related to inflammation through mechanisms potentially overlapping with those altered by physical pollutants and toxicants' (Wright et al 2005). Functional changes in the hypothalamus-pituitary-adrenal cortex-axis are under discussion. Buske-Kirschbaum compiled an overview of the psychobiological aspects of atopic eczema and confirmed by means of hypotheses the various endocrine, immunological and psychophysiological influences on atopic eczema (Buske-Kirschbaum et al. (2001). Recent studies demonstrated also stress-induced alterations in permeability barrier homeostasis, mediated by increased endogenous glucocorticoids (Choi et al 2005) so the existence of a neurogenous inflammation reaction in atopic dermatitis seems to be present.

The influence of serious events in life and of stressors of various degrees on the immune system is known (Kodama et al 1999). The autonomic nervous system acts as the connector between feelings and subsequent somatic response. Lymph nodes contain sympathic afferents; adrenergic and cholinergic fibers are found in the thymus, the lymphocytes also have adrenergic and cholinergic receptors.

Kupfer (1994) examined the interaction between the severity of skin symptoms, the expression of individual emotions and the excretion of salivary cortisol and salivary IgA. Aggression, depression and anxiety were found to be emotions particularly related to skin symptoms. The central immunoetological role of neuropeptides such as NGF and BDNF as well as histamine, acetylcholoin and ECF-A (Eosinophil-Chemotactic Factor of anaphylaxis) in atopic eczema patients were delineated.

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IC4. Anxiety and Atopic Dermatitis

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It has been well documented that emotional and psychological stresses bring on attacks or cause exacerbation of skin symptoms in atopic dermatitis (AD). Recent studies have revealed that stress elaborates anxiety on one hand and affects immune functions on the other. State-Trait Anxiety Inventory (STAI) is a self-administered questionnaire of 20 questions concerning trait anxiety (TA; the anxiety felt in general) and 20 concerning state anxiety (SA; the anxiety felt at present). STAI is scored from a minimum of 20 (the lowest level of anxiety) to a maximum of 80 (the highest level of anxiety) on each scale. With the use of STAI, we have shown that, while having higher anxiety levels than normal individuals, AD patients with stronger perception of TA than SA enhanced serum IgE synthesis. Serum total IgE levels were highly correlated with ratios of TA/SA, indicating that those with higher TA levels compared with SA levels had elevated serum IgE. In accordance with this finding, Th1/Th2 ratios as assessed by interferon-producing cell numbers/IL-4-producing cell numbers were correlated inversely with TA and the ratio of TA/SA. Anxiety did not correlate with the severity of dermatitis, itching or eosinophil number. We suggest that persistent stress stimulates a Th2 immune response in AD through preferential elaboration of TA. Stressors stimulate the hypothalamus-pituitary-adrenal axis and sympathetic nervous system, releasing adrenal glucocorticosteroids and norepinephrin, respectively, and thus tilt cuntaneous inflammation toward the Th2 response.

The patients with AD are suffering from intolerable itching night and day, which is triggered by physiological and psychic stimuli. The patients are not always benefited through antagonizing histamine. We carried out an open trial to examine the effect of tandospirone, a serotonin 1A receptor agonist exerting anti-anxiety effects, 30mg per day for 4 weeks, on relief of skin symptoms in adult AD patients. In the patients with TA/SA of >1.0, the TA/SA ratio significantly decreased when treated with tandospirone compared to without it. Moreover, itching as measured by VAS decreased more significantly in the treated group than the non-treated group among the patients with intense anxiety (TA/SA >1.0 and TA >45). Our findings suggest attenuation of itching by successful control of mental stresses with tranquilizers.

In conclusion, everyday stressors may repeatedly stimulate a Th2 immune response to prevent attenuation of skin inflammation from regulatory immune mechanism, resulting in protraction of inflammatory responses. Intervention with sedatives can be a part of the management strategy in stress-associated itching in AD patients and possibly beyond.

OC12. Intracellular Control of CTACK/CCL27 (Cutaneous T Cell Attracting Chemokine) in Keratinocytes through the Nuclear Transcription Factor Kappa B (NF-kB)

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The CC-chemokine CTACK/CCL27 is highly expressed in inflammatory skin disorders such as atopic dermatitis, psoriasis and contact dermatitis. CTACK binds to the chemokine receptor CCR10 which is highly expressed on skin homing CLA+(cutaneous lymphocyte associated antigen) lymphocytes. CTACK is along with the CC-chemokine TARC/CCL17 (thymus and activation-regulated chemokine) considered as pivotal chemokines in the attraction of lymphocytes to the inflamed skin in atopic dermatitis.

NF-kB is a dimeric protein made up as a hetero- or homodimer from the proteins belonging to the Rel family of proteins (RelA (p65), RelB, cRel, p52 and p50), and plays a vital role in inflammation through control of transcription of genes for chemokines and proinflammatory cytokines.

CTACK production is induced in keratinocytes by TNF-alpha, and it is well known that TNF-alpha is a potent activator of NF-kB. Thus, our hypothesis was that inhibition of NF-kB would also inhibit the TNF-alpha induced CTACK production.

We inhibited TNF-alpha induced CTACK production in keratinocytes using the non-specific NF-kB inhibitors sodium salicylate, 3,4-dichloroisocoumarin, and phenylarsine oxide. To substantiate the results and to further investigate which components of NF-kB are involved in the TNF-alpha induced CTACK production in keratinocytes we incubated the keratinocytes with antisense oligonucleotides against p50 and p65 (three targets each) and found that these potently inhibited CTACK production both alone and together. This indicates that the NF-kB molecule controlling CTACK production is a heterodimer consisting of p50 and p65.

Recent results have shown that deletion of certain IkB molecules, which under normal circumstances retain the NF-kB molecule in the cytoplasm of the skin and inhibit their function, lead to conditions that resemble atopic dermatitis. Although it is still speculative, we suggest that defects in the NF-kB system play a central role in the process leading to inflammation in the skin in atopic dermatitis.

OC13. Brain-Derived Neurotrophic Factor Exerts Immunomodulatory Functions in Atopic Dermatitis

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Recent studies have gained widespread attention to the complex regulation of genetic, environmental, immunologic and pharmacologic factors that contribute to the development of atopic dermatitis (AD). However, neuroimmune interactions of this chronic inflammatory skin disease still remain to be elucidated. The aim of this study was to assess the functional role of the neurotrophin brain-derived neurotrophic factor (BDNF) on highly purified peripheral blood eosinophils of AD and non-atopic subjects.

BDNF receptor expression (p75NTR and trkB) was higher on AD eosinophils compared with that seen in nonatopic subjects ($p<0.05$–0.001). Eosinophil apoptosis was inhibited by BDNF ($p<0.01$–0.01) and chemotactic index was increased ($p<0.001$) in BDNF stimulated AD eosinophils, whereas this effect was not shown in nonatopic subjects. In addition, BDNF levels were increased intracellularly and in supernatants of AD eosinophils compared with nonatopic subjects ($p<0.05$–0.001). Taken together, this study provides the first evidence for a functional role of BDNF on AD eosinophils probably mediated by an increased expression of BDNF receptors. In addition, higher intracellular BDNF levels and the release of BDNF by eosinophils underline the particular importance of BDNF in patients with AD, pointing to new pathophysiologic aspects of this chronic inflammatory skin disase.

OC14. Increased Expression and a Potential Anti-Inflammatory Role of TRAIL in Atopic Dermatitis

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Background: TRAIL, the tumor necrosis factor-related apoptosis-inducing ligand, induces apoptosis of many transformed but also of non-transformed cells. In addition, TRAIL receptor activation has been reported to activate non-apoptotic signaling pathways. This study was aimed to investigate the expression and a potential role of TRAIL in atopic dermatitis (AD).
Patients and Methods: Skin biopsies and/or blood samples were taken from 10 patients with AD and 9 healthy controls. TRAIL expression was detected by flow cytometry and by immunofluorescence staining followed by confocal microscopy. In vitro TRAIL-induced interleukin (IL)-1 receptor antagonist expression by HaCat cells was measured by RNase protection assay and ELISA. IL-1 receptor antagonist expression in AD skin was stained by an immunofluorescence technique.
Results: We observed an increased expression of TRAIL in peripheral blood T cells and monocytes from patients with AD compared to control individuals. High TRAIL expression was also observed in skin-infiltrating T cells of atopic dermatitis patients. Topical tacrolimus treatment reduced the total number of T cells in the skin, but the relative proportion of TRAIL positive cells within both CD4+and CD8+cell populations did not change. TRAIL was demonstrated to induce the expression of interleukin-1 receptor antagonist in keratinocytes in a caspase-independent manner in vitro. Moreover, increased expression of interleukin-1 receptor antagonist was observed in keratinocytes of AD lesional skin.
Conclusion: These data suggest that TRAIL expressing inflammatory skin cells may contribute to the epidermal activation of the interleukin-1 receptor antagonist gene in AD.

OC15. Graphology and Atopic Dermatitis

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Rook and Wilkinson consider the management of emotional factors to be of significance in 40 percent of patients attending departments of dermatology(1). The main types of host factors that can influence stress (genetics, perception) and the numerous types of stressors (environmental, behavioral, psychological) have been studied by dermatologists together with the possible role of neuropeptides in the pathogenesis of some common dermatoses (2). Despite this recognition, systematic study of the interaction and application of principles derived from areas of psychosomatic research are lacking. Clinical experience seems to indicate that for some patients, psychosocial stress is a factor in the precipitation or exacerbation of their illness and in some cases indeed the major factor. Psoriasis, vitiligo, atopic dermatitis (AD) and alopecia areata more than other dermatoses illustrate the subtle interactions between genetic predisposition, environmental influences and psychosocial factors that must take place before the disease become manifest. In particular, recent studies suggest that AD patients with a higher anxiety level are more likely to improve their psychologic and dermatologic condition after psychotherapy, but are more vulnerable to nonadherence when no adequate psychologic treatment is offered (3). Graphology can be a useful tool in spotting health problems before they become too severe, and is excellent at identifying stress in the individual (4). The aim of our study was to detect psychosomatic components in AD through the systematic study of handwriting. The possibility of detecting specific deficiencies, such as physical weakness, poor memory, anxiety, stress, depression and lack of confidence can be identified by graphology. Preliminary results indicate that the quality of handwriting not only can be disturbed in AD patients but the same can be modified in the course of the disease. Despite the fact that some authors question its predictive validity (5), graphology could help therapists identify hidden problems and plan a more specific and tailor-made treatment.

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Friday 16 September 2005

Session 5: Clinical Research, Prognostic and Severity Markers

KL7. A Novel View on the Natural History of Atopic Dermatitis

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Progress in molecular genetics, epidemiology and immunology have provided new tools to study in more depth the pathophysiology of atopic dermatitis (AD). In this context and by using a more stringent definition of atopy and atopic diseases, scientists realized that eczematous diseases looking like AD may be distinguished in at least 2 entities: AD *stricto sensu* and "eczema" (without the context of atopic sensitization, i.e. former intrinsic form of AD) (Johannsson et al, JACI 2004). Most intringingly, positional cloning approach has shown more similarities between gene loci for AD and psoriasis than between AD and asthma. This finding is rather surprising and challenges the concept of atopic march. Moreover, candidate gene approach has unravelled differences between AD and eczema in the incidence of single nucleotide polymorphisms (SNPs) in genes such as IL-4Ra or IL13.

Epidemiological studies revealed that in infants AD-like skin lesions often start in the absence of specific IgE, strongly implying that IgE sensitization is not a prerequisite for eczematous lesions and may occur secondarily to cutaneous inflammation. Whether this inflammatory reaction may be driven solely by e.g. food allergen specific T cells which have been shown to be detectable very early in life remains to be clarified. Moreover, in animal models it has been shown that an OVA specific IgE response, asthma and inflammatory skin lesions can be mounted by repeated topical application. On the other hand, specific IgE towards self-proteins such as structural proteins from keratinocytes have been shown to be common in adults AD patients with highly elevated serum IgE suggesting the possibility of an autoimmune scenario occurring in the context of AD and evolving from mechanisms of molecular mimicry based on initial IgE response to highly conserved microbial structures as it seems to be the case for MnSOD.

Thus, based on these observations a new picture of the natural history of AD emerges where the disease may start as "eczema" and – depending of the gene sets involved – may evolve in a typical AD *stricto sensu*. This is then followed by autosensitization with specific IgE response to self proteins. However, the pathophysiological significance of these IgE remains to be proven. If this turns to be the case, autosensitization may explain why prevention measures such as allergen avoidance are less efficient in patients with a longer history of AD. This – admittedly speculative – view of the natural history of AD should be verified in the context of birth cohorts and ultimately enforce the concept of early intervention and the development of disease modifying strategies.

IC5. Autoreactivity in Atopic Dermatitis—Induced by Skin Fungi? IgE- and T Cell Mediated Autoimmunity Against Manganese Superoxide Dismutase in Atopic Dermatitis

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Autoreactivity to human proteins has been postulated as a decisive pathogenetic factor for patients with atopic dermatitis (AD) on the basis of the detection of IgE directed against various proteins in vitro. In this study, an essential, stress-inducible enzyme—human manganese superoxide dismutase (hMnSOD), known for its autoreactivity in ABPA—was found to act also as an autoallergen in a subset of patients with AD, clearly demonstrated by eczematous reactions through atopy patch tests with the application of recombinant hMnSOD. Human MnSOD also induced positive skin prick test results in 29 of 69 patients with AD in addition to IgE- and T cell–mediated in vitro reactivity. Interestingly, such reactivity was also found in patients with nonatopic eczema and strongly correlated with disease severity. All patients reacting to hMnSOD were sensitized against the skin-colonizing yeast Malassezia sympodialis, known for its pathogenetic role in AD. MnSOD of fungal origin and MnSOD of human origin show a strong structural relationship. Thus, sensitization is most likely induced by exposure to environmental fungal MnSOD of M sympodialis, resulting in molecular mimicry due to secondary autoreactivity with its human counterpart. Further studies will have to elucidate now whether consequent anti-inflammatory or antifungal treatment might prevent such potentially harmful phenomena in AD.

OC16. Expression of Thymic Stromal Lymphopoietin (TSLP) in Keratinocytes of Atopic Dermatitis Patients and Normal Controls

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Human thymic stromal lymphopoietin (TSLP) is a novel IL-7-like cytokine produced by human epithelial, stromal, and mast cells. TSLP-activated human DCs produce Th2-attracting chemokines such as TARC and MDC but not IL-12. TSLP-DCs induce the generation of CD4 + Th cells with a pro-allergic phenotype and also induce the differentiation of CD8 + T cells into IL-5 and IL-13-producing cytolytic effector cells. It has been reported that TSLP is highly expressed in the lesional keratinocytes of atopic dermatitis but not in the non-lesional keratinocytes of atopic dermatitis and other types of disease with skin inflammation. We performed our study to verify the differential expression of TSLP in keratinocytes among lesional, non-lesional sites of atopic dermatitis and normal control. We also observed the expression of TSLP in keratinocytes treated with various cytokines. Our study shows that TSLP is expressed all by keratinocytes of normal controls, lesional and non-lesional sites of atopic dermatitis patients. However, TSLP is much highly expressed in lesional keratinocytes of atopic dermatitis than in non-lesional and normal keratinocytes using confocal laser microscopy and immunohistochemistry methods. TSLP expressions did not differ among keratinocytes treated with IL-4, TNF-alpha, TGF-beta and KGF.

OC17. Identification of *Malassezia Sympodialis* in Patients with Atopic Dermatitis by Polymerase Chain Reaction and its Impact on Disease Activity

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Background: In recent years Malassezia sympodialis has received particular attention in the pathogenesis of atopic dermatitis (AD). The existing evidence suggests that both IgE- and T-cell mediated immune reactions could be involved. However, investigations to demonstrate the significance of colonization with this yeast and sensitization to it are scarce since Malassezia spp. are more difficult to isolate and culture than other pathogenic yeasts.

Objective: This study was aimed at the identification of Malassezia sympodialis in patients with AD by establishing a DNA-based procedure directly applicable to pathological skin scales, at the sensitization rate against Malassezia sympodialis among AD patients and the yeast's influence on disease activity.

Methods: Sera from 52 patients suffering from AD and 10 healthy controls were investigated for specific IgE against Malassezia sympodialis and the scoring index (SCORAD) of all AD patients assessed. In addition, Malassezia sympodialis DNA was extracted from skin scales of three different localizations by a modified hexadecyltrimethylammonium bromide (CTAB) method and amplified by single polymerase chain reaction (PCR), using the general fungal ITS 1/4 primers for amplification of sequences from the Malassezia major ribosomal DNA complex.

Results: Malassezia-specific IgE were positive in 24/52 patients (46.2%) and in none of the healthy controls. DNA could be extracted from skin scales of 36 patients (69.2%), mainly from affected skin scales, less from the neck and unaffected skin. Only 2/10 (20%) of the healthy controls showed colonization with the yeast. There was no significant correlation between SCORAD and colonization with Malassezia sympodialis, SCORAD and sensitization rate or sensitization rate and colonization ($p > 0.05$).

Conclusions: These results eviscerate the role of Malassezia sympodialis in AD since there is no clear explanation for the occurrence of specific IgE without colonization or increased SCORAD and vice versa. However, this finding may also imply a support from adjuvant factors in the sera or skin of AD patients. The importance and relative contribution of Malassezia sympodialis sensitization to AD remains to be established.

OC18. High Concentrations of Circulating Macrophage Migration Inhibitory Factor in Patients with 'Extrinsic' Atopic Dermatitis

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Background: The most employed diagnostic criteria of atopic dermatitis (AD) can be fulfilled in the absence of elevated total circulating IgE or specific IgE to food allergens or environmental aeroallergens and in the absence of personal or familial history of atopy as well. Therefore a distinction between 'extrinsic' or 'allergic' and 'intrinsic' or 'non-allergic' AD has been suggested. Macrophage migration inhibitory factor (MIF) is a pleiotrophic lymphocyte and macrophage cytokine; it is likely to play an important role in innate immunity. Serum MIF content was significantly elevated in patients with AD and PBMCs should be an important source of increased serum MIF in AD.
Objective: This study was designed to investigate the differences of plasma MIF levels between 'extrinsic' and 'intrinsic' AD and the correlation between plasma MIF levels, total plasma IgE levels and house dust mite (HDM) allergen-specific IgEs.
Methods: Plasma MIF levels were measured by an enzyme-linked immunosorbent assay in 116 healthy controls and 265 AD. The plasma levels of the total and HDM allergen-specific IgEs were measured by a latex photometry immunoassay and enzyme-linked immunoabsorbent assay in patients with AD. Differences between groups were nonparametrically statistical analysis by using Kruskall-Wallis test.
Results: Plasma MIF and log [total IgE] levels were significantly increased in AD patients compared with control subjects ($P = 0.0028$ and $P < 0.0001$, respectively). Plasma MIF concentrations in atopic dermatitis patients were significantly positively correlated with the specific IgE D.farinae score ($r = 0.17$, $P < 0.01$). The differences of MIF levels between the two AD subgroups and control group were statistically significant ($P = 0.0005$).
Conclusion: Increased MIF levels are associated with AD symptoms and MIF may become a useful clinical parameter to understand the 'extrinsic' AD.

OC19. Elevated Serum Levels of I-309/CCL1 in Patients with Severe Atopic Dermatitis

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Background: Th2 lymphocytes, which play a critical role in the pathogenesis of atopic dermatitis (AD), employ the CC chemokine receptors CCR4 and CCR8 with the legands thymus- and activation-regulated chemokine (TARC/CCL17), macrophage-derived chemokine (MDC/CCL22) and I-309/CCL1. It has already been reported that TARC/CCL17 and MDC/CCL22 were elevated in sera from patients with AD. There has been one recent report of I-309/CCL1 in the pathogenesis of AD patients (J Immunol. 2005 174:5082-91).
Objective: The aim of this study was to clarify the participation of I-309/CCL1 in the pathogenesis of AD.
Methods: Forty-seven patients aged 18 to 64 years with mild to severe AD and 9 healthy volunteers aged 26 to 45 years were examined. Disease severity was determined by Rajka & Langeland grading. Thirty-eight patients were subdivided into three groups by disease severity: mild (score 3–4; $n = 16$), moderate (score 4.5–7.5; $n = 17$) and severe (score 8–9; $n = 5$). The serum I-309/CCL1 level was measured through the use of ELISA. The limit of detection was 0.7 pg/ml. The relationships between I-309/CCL1 levels and various laboratory values, such as lactate dehydrogenase (LDH), IgE, eosinophil numbers in peripheral blood, and eosinophil cationic protein (ECP) were analyzed. Immunohistochemical staining of a skin section with anti-I-309/CCL1 Ab was performed using the avidin-biotin-alkaline phosphatase system as a preliminary study.
Results: The serum I-309/CCL1 levels of patients with AD and of control subjects were $4.21 +/- 4.88$ pg/ml and $0.62 +/- 0.67$ pg/ml (Mean $+/-$ SD), respectively. There was a statistically significant difference between the AD and control subjects ($p = 0.0007$). We compared the serum I-309/CCL1 levels among the 3 groups of patients with AD. The I-309/CCL1 levels in patients with severe AD were significantly higher than in patients with mild AD ($p = 0.0288$), but were not higher than those with moderate AD. The serum I-309/CCL1 levels correlated with eosinophil numbers in peripheral blood ($r = 0.529$) and LDH ($r = 0.617$), whereas the serum IgE levels weakly correlated with I-309/CCL1 levels ($r = 0.47$). We evaluated the serum I-309/CCL1 levels in 10 patients with AD during the clinical course. Disease severity decreased in all patients after the treatment ($p = 0.0051$). However, serum I-309/CCL1 levels did not decrease after the treatment. Specifically, serum I-309/CCL1 levels increased after the treatment in 2 patients who had high serum I-309/CCL1 levels before the treatment. The preliminary results of immunohistochemical analysis showed that dermal endothelial cells markedly expressed I-309/CCL1 protein in lesional atopic skin in comparison with normal skin.
Conclusion: Elevated levels of I-309/CCL1 were clearly shown in the serum of patients with AD in comparison with the levels in control subjects, and it was demonstrated that the levels of I-309/CCL1 correlated with the serum LDH levels, which are reported to represent the disease severity of AD. Although there was actually a very small amount of I-309/CCL1 compared with TARC/CCL17 in the serum of AD patients, we precisely detected I-309/CCL1 in the serum from AD patients and demonstrated a correlation between the level of I-309/CCL1 and the LDH level or the eosinophil numbers in peripheral blood. We concluded that serum I-309/CCL1 levels could be one of the markers of disease severity for AD.

OC20. Serum Levels of IL-16 and Disease Activity in Children with Atopic Dermatitis

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Background: Il-16 is a natural ligand of CD4 molecules and induces chemotaxis in CD4-expressing cells. It amplifies the inflammatory reaction by stimulating cytokine production in monocytes and activating T-cells. Evidence has been accumulated that IL-16 plays a role in the pathogenesis of atopic dermatitis and increased serum levels of IL-16 have been detected in autoimmune and allergic diseases. However few data are available on IL-16 serum levels in atopic dermatitis. (1,2)
Aim: to evaluate IL-16 serum levels in childhood atopic dermatitis before and after treatment in order to obtain data of a possible correlation between IL-16 serum levels and other clinical markers of disease severity and activity.
Methods: We measured by an ELISA approach, IL-16 serum levels in 34 children (18 males and 16 females; mean age 6,5 years), with moderate to severe atopic dermatitis at their first visit and after 3 months of treatment and in 10 non atopic sex and age-matched healthy controls. In the children with atopic dermatitis we also evaluated other clinical markers of disease activity (e.g. SCORAD index, serum IgE values, peripheral eosinophil counts).
Results: IL-16 serum levels were significantly higher in patients affected by atopic dermatitis than in controls at the beginning of the observation, as well as after 3 months of treatment with tacrolimus. However no clear correlation was found between IL-16 serum levels and the other clinical markers.
Conclusion: Our preliminary results, while showing increased levels of serum IL-16 in children with atopic dermatitis, seem to raise some doubts on the use of IL-16 serum level as a marker of disease activity in childhood atopic dermatitis.

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OC21. Effect of Caring for a Child With Atopic Dermatitis and Asthma on Parental Sleep, Depression and Anxiety Scores: A Prospective Comparative Study

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Objective: To compare the relative impact of caring for a child with atopic dermatitis and asthma on parents' sleep and level of anxiety/depression.
Design: Prospective, descriptive, questionnaire-based study.
Setting: Regional specialist eczema and asthma outpatient clinics at a tertiary paediatric hospital in the UK.
Participants: 91 parents (55 mothers and 37 fathers) with children who had moderate to severe atopic dermatitis or asthma, but not both.
Main outcome measures: Number of sleep disturbances and minutes the mother and father were up with their child during the previous two nights. Mothers' and fathers' hospital anxiety and depression scale scores.
Results: Mothers caring for children with moderate to severe atopic dermatitis lost a median of 39 minutes of sleep/night and fathers lost 45 minutes sleep/night. This compared with a median of no sleep lost by parents who had children with asthma ($P < 0.001$). Multi-variant analysis showed that these results were independent of the age of the children. Within these families, there was a direct correlation between the severity of sleep disturbance and the level of maternal depression ($rho = 0.73$; $P < 0.001$) and anxiety ($rho = 0.58$; $P = 0.002$), as well as with the level of paternal anxiety ($rho = 0.59$; $P = 0.01$).
Conclusions: Compared with looking after a child with chronic asthma, caring for a child with chronic atopic dermatitis was associated with significantly higher parental sleep disturbances. Sleep disturbances correlated strongly with the parental anxiety levels, and in the case of mothers, depression scores.

OC22. Flare Cycles, Itch-scratch Loops and Associated Downturns in QoL: The Human and Economic Burden of Atopic Dermatitis on Patients and Caregivers

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Objective: Atopic dermatitis (AD) is a chronic, cyclical, relapsing, inflammatory skin condition. Flare cycles, development of visible skin lesions and aggravation of the 'itch-scratch-cycle' during acute flare-ups of AD form the basis for significant impact on quality of life for children and their caregivers as well as for adult patients. The aim of this study is to gather information on the human and economic impact of AD and to examine which factors are the most important determinants of QoL of patients and caregivers.

Method: ISOLATE is the first large-scale, international survey to assess the impact of AD on the patients, care-givers and society. A total of 2,002 patients (>13 yrs) and caregivers of children (2–13yrs) with moderate to severe AD from 8 countries were surveyed. Beside other instruments PIQoL-AD, QoLIAD, EQ-5D and an assessment of work productivity loss are included in the study. Multiple regression are used to evaluate which factors significantly impact QoL scores and the cost of illness are calculated. Cross-sectional QoL scores are evaluated between demographic groups and according to disease-related factors; age groups are created based on distributions to enable statistical analyses; continuous variables were grouped to facilitate statistical tests; correlations were calculated (Spearman Rank) and non-parametric tests for independent samples (Mann-Whitney U Test for two groups or Kruskal-Wallis One-way Analysis of Variance for three or more groups) are employed.

Results: The QoL of AD-sufferers is mainly affected by unhappiness and depression due to flare-up. The most important other factors for QoLIAD are percentage of performance at work affected, percentage of body affected, severity of itch, severity of eczema and the number of nights woken during flare-up. 33.6% of the variance is accounted for by this model, of which 20.8% is represented by how unhappy/depressed the flare-up made. Downturns in QoL of caregivers are primarily due to the nights woken during flare-up. Additional important explanatory variables are percentage of body affected, percentage of performance at school affected, unhappiness/depression due to child's flare-up, bright redness of AD, concerns over eczema treatments and days taken off school. 27.8% of the variance is accounted for by this model, with 10% of this variance represented by the number of nights that the child is woken due to having a flare-up.

Patients report mean utility values of .783, caregivers of .79. Statistical significant between group differences exists for severity of the disease, days of work (patients and caregivers), hospitalization, percent of work affected (caregivers) and number of nights sleep affected (patients).

Costs of AD are mainly driven by social costs due to absenteeism and presenteeism and direct medical costs. The impact of AD on labour market costs and social welfare loss is significant.

P7. Atopic Dermatitis & the Adolescent Patient

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Background: Visible skin lesions and intense itching significantly impact the quality of life of patients with AD. Adolescents are a particularly vulnerable group of AD patients as they are at a critical life stage where personal appearance, interaction with peers and educational performance begin to matter. ISOLATE is the first international, large-scale survey to provide insight into how adolescent patients view the impact AD flares have on their lives and their attitude towards current treatment options.

Methods: 2,002 patients (>13 yrs) or their caregivers (children 2–13 yrs) with moderate to severe AD, from 8 countries underwent in-depth telephone interviews utilising a comprehensive questionnaire developed in collaboration with national eczema patient groups and physicians. A flare was defined as inflammation of the skin requiring a physician consultation or application of prescription medication. The results for the (n = 125) adolescent patients (14-17 yrs) are reported here.

Results: Adolescent AD patients experience on average 8 flares and spend more than 3.5 months in relapse each year. The flare causes extreme itching in 60% of adolescent patients and prevents 83% of them from participating in everyday activities. ISOLATE demonstrates how the stigma of AD can affect the adolescent patient's mood, self-confidence and ability to conduct relationships. While in flare 70% of adolescents are embarrassed about their appearance; 52% feel unhappy or depressed; 36% agree that their self-confidence is affected; 23% worry that it will make it more difficult to form relationships with a boyfriend or girlfriend and 53% are either always or sometimes worried about their next relapse.

Despite the emotional impact of AD on adolescent patients, 66% have not discussed their feelings with their physician. 46% of adolescent patients report that the flare has an effect on their school life and 39% have been teased or bullied due to their AD. 41% of adolescent patients agree that during a flare their concentration is affected while at school, which may partly be explained by disturbed sleep experienced for an average of 12 out of 15 nights per flare. The majority of adolescent patients are prescribed reactive use of topical corticosteroids (TCS) to control the AD flare, however, 51% are concerned about the use of these agents due to side effects and 65% use TCS only as a last resort. 68% of adolescent patients stated that being able to effectively control their AD would be the single most important improvement to their quality of life.

Conclusions: ISOLATE demonstrates the significant impact AD has on adolescent patients quality of life; the strong desire they have to gain control over their condition; their concern about the use of TCS treatment regimens and the urgent need to improve long-term management strategies for this vulnerable group. *This survey was supported by an educational grant from Novartis.*

P6. Comparative Efficacy of Hanifin and Rajka's Criteria and U.K. Working Party's Diagnostic Criteria in Diagnosis of Atopic Dermatitis in a Hospital Setting

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Background: Diagnosis of atopic dermatitis depends on clinical features as no definitive diagnostic test exists. Hanifin and Rajka's criteria were acceptable for hospital based studies but were found not to be suitable for field studies. U.K. working party formulated a clinical diagnostic criteria that could be used both in hospital and epidemiological setting. Validation studies of the criteria showed widely variable results, probably due to different clinical settings and ethnicity.

Aim and objective: This study was undertaken to validate Hanifin and Rajka's criteria and to assesss the comparative efficacy of Hanifin and Rajka's criteria and U.K. working party's diagnostic criteria in diagnosis of atopic dermatitis in a hospital setting in North India.

Subjects and methods: This study serially included 101 patients of atopic dermatitis and 48 controls of paediatric age group. The study period was from July 2003 to December 2004.

Results: Hanifin and Rajka's criteria (senstivity- 96%, specificity- 93.75%, positive predictive value- 97% and negative predictive value- 91.84%) had statistical advantage over U.K. working party's diagnostic criteria (sensitivity- 86%, specificity- 95.83%, PPV- 97.75% and NPV- 76.67%) with p-value <0.005.

Session 6: Animal Models

KL8. Canine Atopic Dermatitis: A Natural Model to Study the Human Disease
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For decades, a pruritic skin disease diagnosed as atopic dermatitis (AD) has been identified in dogs living in a natural environment. This disease usually occurs first in juveniles or young adults, and there is a clear breed and family predisposition. Limited data suggest that the prevalence of canine AD may be increasing. Canine AD may occur in association with allergic rhinitis and conjunctivitis, but signs of asthma are uncommon. The most common allergenic flare factors for canine AD are of environmental – especially Dermatophagoides farinae house dust mite – and dietary origin. Clinical signs of canine AD include erythema and secondary skin lesions that occur predominantly on the face, paws, axillae, groin, perineum and flexural aspects of the limbs. Dogs with AD exhibit common cutaneous infections with either Staphylococci or Malassezia organisms. There is good evidence suggesting that topical or oral glucocorticoids and the calcineurin inhibitors cyclosporine and tacrolimus are the most effective to alleviate signs of AD in dogs. Immunologically, canine AD is usually associated with high serum levels of allergen-specific IgE, but such reaginic antibodies are absent from rare individuals. Additionally, skin lesions of canine AD resemble those of its human counterpart at both cellular and mediator levels. In both spontaneously or experimentally sensitized dogs, the epicutaneous application of allergens to which the subjects are hypersensitive triggers an inflammatory reaction that mirrors lesions of natural AD. In summary, the epidemiology, clinical signs, treatment outcome and immunological aberrations of canine AD make this spontaneous dog model relevant to study the pathogenesis of AD or test novel interventions to prevent or treat this disease in humans.

IC 6. Spontaneous Dermatitis in Mice Transgenic for Human Apolipoprotein C1
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Mice with transgenic overexpression of human apolipoprotein C1 (APOC1) in liver and skin have strongly increased serum levels of cholesterol, triglycerides and free fatty acids, indicative for a disturbed lipid metabolism.

Importantly, these mice spontaneously develop symptoms of dermatitis from an age of 7 weeks on including pruritus, erythema and lichenfication. Pruritus was quantified by an accelerometer and this revealed significantly increased scratching in APOC1 mice as compared to wild-type mice; the scratching intensity increased during aging of these mice. Also these mice show a loss of skin-barrier function evident from increased Trans Epidermal Water Loss (TEWL). Histological analysis show increased epidermal thickening together with elevated numbers of inflammatory cells in dermis and epidermis.

Daily topical treatment with triamcinolone acetonide (0.1% in cream) for a period of 3 weeks, starting at an age of 9 weeks, significantly reduced several aspects of dermatitis, as demonstrated by reduction of the pruritus and decreased epidermal thickening. These data indicate that the APOC1 transgenic mouse represents a spontaneous model of dermatitis that is sensitive to treatment with corticosteroids. Our current research focuses on the validation of the APOC1 transgenic mouse as a model of (allergic) atopic dermatitis.

OC23. Establishment of a Mouse Model for Atopic Dermatitis: Getting New Insights into the Role of T Cells
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Mite antigens play important roles in the onset and/or development of atopic dermatitis, and mite antigen-induced dermatitis model appear beneficial for the basic study of atopic dermatitis. Therefore to understand the onset and development of the disease appropriate animal models are essential. Recent studies on eczematous skin diseases such as atopic dermatitis have shown the role of T cells as effectors of the manifestation of the eczema. In the current study, we attempted to establish an allergic dermatitis model in normal C57Bl6 mice using as antigen Dermatophagoides farinae (DF) as antigen in order to study the T-cell-mediated immune effector mechanisms. Mite antigen solution was applied epicutaneously onto the ear 3 times per week and then the mice were let to rest for another week (cycle of two weeks). Several cycles were done. After 4 cycles we observed thickening of the epidermis of the ear and infiltration of the skin by eosinophils and T cells as demonstrated by immunohistological and RT-PCR analysis. Levels of total IgE were mildly increased (factor 2). Furthermore, DF-specific splenocytes and draining lymph nodes cells (as well as CD8 + T cells) were isolated from lymph nodes and spleens of sensitised mice. In an alternative model, sensitization of naïve mice was obtained by s.c. injection of bone marrow dendritic cells (BMDC) charged with DF. Seven days later, mice were challenged by a topical application of DP onto the ear and the ear swelling was measured. This model offers the advantage of a quicker sensitization (7 days instead of 41 days) and the possibility of depleting selectively discrete T cell populations and study the impact to the development of the DF-induced dermatitis.

OC24. Collared Mice: A Model to Assess the Effects of Scratching
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There is no current in vitro or in vivo method to easily assess itching despite its clinical importance in various skin diseases, such as atopic dermatitis, in which itching is a primary or secondary symptom. The purpose of this study was to determine the effect of scratching on the development of contact hypersensitivity (CH), an experimental model of allergic skin reactions, and to assess itching as a physiological response using a murine model of CH. We utilized plastic collars which were placed around the neck to prevent the mice from scratching (or grooming) their ears during CH. Following elicitation, the ear swelling of collared mice was significantly decreased, by 41–51%, compared to control mice in which collars were not used. Thus, we conclude that scratching contributes considerably to the increase in ear thickness that is seen in CH in mice. This model thus enables us to assess itching as a part of the CH skin reaction, and may be suitable to assess or screen anti-pruritic agents or new drugs. We therefore assessed the effect of various agents like antihistamines (chlorpheniramine), steroids (prednisolone), non-steroidal anti-inflammatory agents (aspirin) and sedative agents (phenobarbital) on CH, and found that all of these agents decreased CH (by approximately 36–54%, depending on the time between elicitation and drug administration) when collars were not used. Notably, sedative agents had anti-pruritic properties without any anti-inflammatory effects, since they had no apparent effect on CH in collared mice, while the other anti-pruritic or anti-inflammatory agents caused 31–60% decreases of CH in the collared mice. We also explored for factors involved in the decrease of CH in collared mice using Affymetrix Genechip technology. Among 39,000 genes, a total of 135 genes were consistently and differentially regulated (124 up- and 11 down-regulated) during CH in collared mice as compared to controls; 2 immune-response genes were downregulated and 7 immune-modulating genes were upregulated in collared mice. This model thus enables us to better dissect out the various elements responsible for the elicitation of CH without the confounding scratching that occurs during these reactions.

OC25. Rapid and Specific Acoustic Analysis of Itch in AD Model Mouse
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Scratching is an essential and skin specific behavior resultant from itch – a common symptom for acute and chronic dermatitis, especially atopic dermatitis (AD). Close association between itch sensation and scratching, measurement of the scratching times has been used for evaluation of the itching in animals. Manual counting of the visual images of the experimental animals has difficulty in accuracy and reproduction. Recent introduced digital image analysis for mouse scratching needs handling of huge sized records with manual correction for blind angles. To measure scratch behavior of the atopic dermatitis models, we have developed a novel acoustic measuring system. AD model mice (IL-18Tg) develop scratching sounds, which were digitally recorded and analyzed using novel computer software we have developed. The mice scratched over 20 times/sec., which was untraceable by manual counting. This system counted within a few minutes the scratching times of the model mice recorded for a hour, and clearly revealed in vivo effects of anti-allergic drugs. Different from visual recording, this system does not need strong illumination. Acoustic measurement enables long time recording of the mice scratching under less stressful conditions, and is a potent tool for the development of new therapies for AD.

Session 7: Skin Barrier

KL9. Netherton Syndrome as a Model for Skin Barrier Dysfunction

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Epidermal proteases play a major role in the differentiation and the desquamation processes of the epidermis. The recent identification of SPINK5 encoding the serine protease inhibitor LEKTI as the defective gene in Netherton syndrome (NS) provided the first exemple of a human skin disease caused by defective inhibition of epidermal serine proteases. We generated Spink5-null mice which faithfully replicate key features of NS, including abnormal desquamation, impaired keratinization, hair malformation and a skin barrier defect. LEKTI deficiency in mice causes *Stratum Corneum* Tryptic Enzyme (SCTE) and *Stratum Corneum* Chymotryptic Enzyme-like (SCCE) hyperactivity, resulting in desmoglein 1 degradation and abnormal desmosome cleavage in the upper granular layer. This leads to defective stratum corneum adhesion and resultant loss of skin barrier function, from which the animals die within a few hours of birth. Profilaggrin processing is increased, loricrin and involucrin are overexpressed, implicating LEKTI in the cornification process. Intercellular adhesion is lost in the inner root sheath and hair shafts are irregular and shrunk. Transplantation experiments of whole skin from Spink5 knock-out mice reproduce major histological changes similar to NS patients: the epidermis is hyperproliferative, showing acanthosis, papillomatosis and hypogranulosis, while the stratum corneum is thicker and shows detachment; hair follicles display a pronounced disorganisation and an inflammatory infiltrate is present in the papillary dermis. This work identifies LEKTI as a key regulator of epidermal protease activity, with profound effects on epidermal desquamation, the cornification process and hair formation.

IC7. Stratum Corneum pH Regulates Permeability Barrier Homeostasis

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Providing the interface with a desiccating external milieu, the primary function of the stratum corneum (SC) is to limit excess transcutaneous water loss from the aqueous interior. Whereas the acidic surface mantle of the skin was first described over a century ago, other than its putative antimicrobial activity, its functions remain largely unknown. At least three endogenous mechanisms: a) free fatty acid generation from phospholipid hydrolysis, b) a sodium-proton antiporter (NHE1), and c) histidine metabolism to urocanic acid, acidify either whole stratum corneum (SC), inner vs. outer SC, or specific membrane microdomains. NHE1 localizes to the outer nucleated cell layers and its expression is regulated by changes in surface surface pH independtly from barrier status. Unlike most epithelia, epidermal NHE1 expression increases by SC neutralization and is downregulated by lowering SC pH. Acidification, however, regulates the key SC functions: permeability barrier homeostasis, integrity/cohesion, and antimicrobial activity. We have recently shown that permeability barrier recovery is delayed by even short application of superbases, compounds that are 10 times more basic than in 1N sodium hydroxide. The pH-dependent mechanism that was found to be responsible for the barrier abnormality involves the requirement of an acidic pH for the maximal catalytic activity of certain lipid processing enzymes; e. g., β-glucocerebrosidase (βGlcCer'ase) and acidic shyngomyelinase (aSMase). In contrast to transient pH alterations, sustained SC neutralization altered not only barrier recovery kinetics, but also basal barrier function. These barrier abnormalities were attributable to decreased âGlcCer'ase and aSMase catalytic activity due to serine protease (SP)-mediated enzyme degradation, normalized by co-applied SP inhibitors (SPI). In addition, either: a) short-term exposure of intact skin to neutral pH buffers b) elevation of SC pH by blockade of an endogenous acidifying mechanism or c) short- and long-term applications of superbases compromise SC integrity/cohesion, attributable to accelerated corneodesmosome (CD) degradation. These deleterious effects were also further linked to SP activation, because co-applied SPI normalized both SC integrity/cohesion and CD degradation, even in the face of an elevated pH. Sustained elevations in SC pH are an appropriate model for neonatal skin, which displays normal basal barrier function, but abnormalities in both barrier recovery and SC integrity/cohesion. In addition, many skin diseases, including psoriasis, atopic dermatitis, and other eczemas are associated with prolonged increases in SC pH. One could speculate that such a sustained increase in SC pH could either trigger, exacerbate, or prolong the manifestations of these disorders. In these clinical situations, the pH-induced decrease in either SC integrity/cohesion and/or permeability barrier homeostasis, could further aggravate disease-specific disturbances in SC function.

IC8. Kallikreins in the Stratum Corneum

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In our group, our main interest for the last 20 years has been to understand the process of desquamation. To keep the skin barrier intact, it is very important to have a well regulated proteolysis at the skin surface that is in balance with the de novo production of keratinocytes at the basal cell layer in stratum corneum. This balance is often disturbed in different skin disorders like atopic dermatitis and psoriasis for example. We have now biochemically purified three different serine proteases in active form from the outermost parts of stratum corneum. All three enzymes, stratum corneum tryptic enzyme (SCTE or kallikrein 5), stratum corneum chymotryptic enzyme (SCCE or kallikrein 7) and kallikrein 14, belong to the same gene family, the tissue kallikreins. Both kallikrein 5 and 7 are thought to be involved in the desquamation process. They show a similar expression pattern in the outermost parts of the stratum corneum, and they have been shown to be able to degrade desmosomes at physiological pH. The function of kallikrein 14 is not known, but it is a protein with high tryptic activity. The expression pattern of kallikrein 14 is different compared to the other two proteins. Immunohistochemistry shows predominant staining of the sweat glands. All three proteins can be detected on the outermost surface by analysis of the protein content of tape strips.

All three enzymes are produced as inactive prepro-enzymes that are exported to the outside of the cells. To become active, a proteolytic cleavage by an enzyme with tryptic cleavage specificity of the pro-peptide is required. We have shown that kallikrein 5 can activate itself as well as kallikrein 7 and 14 in a pH dependant manner that may have physiologic implications. Most of kallikrein 14 that is found in the stratum corneum is in its activated form, while quite a large proportion of both kallikrein 5 and 7 can be found as inactive pre-forms.

OC26. Epicutaneous Sensitization to Aeroallergens in Infantile Atopic Dermatitis: Determining the Role of Epidermal Barrier Impairment

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Introduction: As already shown by our group, epicutaneous sensitization to atopens overlaps the onset of atopic dermatitis (AD) in infancy (1). Early epidermal barrier impairment may facilitate the epicutaneous penetration of atopens. The aim of our study was to investigate possible correlations between TEWL measures and aeroallergen sensitization tests in infants with AD.

Patients and methods: This cross-sectional study was carried out in our out-patient unit from May 2002 to June 2004, in 3 to 12 month-old children with a diagnosis of moderate to severe AD (UK working party criteria, SCORAD >15). After written consent, the following tests were performed: TEWL (g/m^2/h) on non-involved skin (TM210 Monaderm^R), specific IgE, atopy-patch test (APT) and skin-prick tests for 7 aeroallergens, i.e., *Dermatophagoides pteronyssinus*, *D. farinae*, cat, dog, birch-pollen, ambrosia, and cockroach. Only APTs with skin readings of $++$ or more were considered positive. A control group of 29 infants without AD was enrolled to validate TEWL measures. Environment was assessed by a questionnaire, and house dust mite (HDM) concentration was measured on dust samples (Acarex-test^R).

Results: 89% of AD infants had positive APT to common aeroallergens. With a mean of 27.4 g/m^2/h, AD infants had a significant higher TEWL than controls (11.1, $p<0001$). Children with at least two positive APT had higher TEWL (29 g/m^2/h) than those with only one positive APT (20.75, $p<0.035$). Moreover, TEWL increased with AD severity, with a mean TEWL of 34.4 in children with SCORAD >40 and 23.7 in children with SCORAD <40 ($p<0.05$). No correlation was found between indoor APT results and exposure to HDM, cat and dog at home.

Discussion: Our study confirms a high prevalence of delayed sensitization to in- and out-door aeroallergens in AD infants. It also confirms in infants a positive correlation between AD severity (SCORAD) and epidermal barrier impairment (TEWL) previously noted by Seidenari et al. in older children [2]. Since we showed that the higher the TEWL, the higher the prevalence of sensitization to aeroallergens, our data argue for an early and provocative role of a constitutive epidermal barrier impairment for atopen sensitization in infantile AD.

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2. Seidenari S, Giusti G. Objective assessment of the skin of children affected by atopic dermatitis: a study of pH, capacitance and TEWL in eczematous and clinically uninvolved skin. *Acta Derm Venereol* 1995; 75: 429–433.

OC27. Re-Characterization of the Non-Lesional Skin in Association with Barrier Function and the Severity of Atopic Dermatitis

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The relationship between the severity of atopic dermatitis (AD) and defects in the barrier function of non-lesional skin of patients with AD is still unclear. To determine whether disrupted barrier function in the non-lesonal skin is associated with the severity of AD and/or with local dry skin properties such as dryness/scaling/itchiness, we evaluated the barrier function and the water content of non-lesional forearm skin and compared that with the severity of AD and the intensity of dryness/scaling/itchiness at the same skin sites. The transepidermal water loss (TEWL) significantly increased in proportion to the severity of AD with a high correlation coefficient ($r = 0.834$, $p < 0.0001$, $n = 106$), while the capacitance decreased in proportion to the severity of AD with a lower correlation coefficient ($r = -0.720$, $p < 0.0001$, $n = 106$) compared with TEWL. Comparison with dry skin properties revealed that while the capacitance values were highly correlated with dryness ($r = -0.752$, $p < 0.0001$, $n = 106$) and with scaling ($r = -0.697$, $p < 0.0001$, $n = 106$), the TEWL was also related to dryness ($r = 0.788$, $p < 0.0001$, $n = 106$) with a higher correlation coefficient compared with capacitance and to scaling ($r = 0.697$, $p < 0.0001$, $n = 106$). Itchiness was more dependent on capacitance rather than TEWL although they had similar correlation coefficients ($r = -0.653$ for capacitance and $r = 0.660$ for TEWL, $p < 0.0001$, $n = 106$). While there was a minor correlation ($r = -0.268$, $p < 0.028$, $n = 72$) in the non-lesional skin of AD between TEWL and capacitance, our results suggest that the abnormal barrier functions of non-lesional skin in AD patients predominantly reflect the severity of AD and are also associated with the intensity of the dry skin properties in concert with the water condition.

OC28. Re-Evaluation of the Importance of Barrier Dysfunction in the Non-Lesional Dry Skin of Atopic Dermatitis: Analysis by Topical Application of a Barrier Cream

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Atopic dermatitis (AD) can be considered a barrier disease in which antigens and irritants that can easily penetrate clinically normal, non-lesional skin due to its defective barrier function trigger and worsen the dermatitis. Thus, replenishing the barrier function in clinically normal, non-lesional skin of patients with AD seems to be a key for preventing the refractory nature of the dermatitis. To determine whether the disrupted barrier function of AD non-lesional skin can be repaired by topical application of a synthetic ceramide known to induce barrier recovery and to subsequently evaluate the relationship between enhanced barrier function and improved dry skin conditions, we applied topically a synthetic ceramide (CER) or hirudoid-(HIRU) containing cream to the non-lesional skin of AD patients for 4 weeks and evaluated their efficacy by measuring trans-epidermal water loss (TEWL) and capacitance values as well as clinical scoring for scaling/dryness/itchiness. Treatment for 4 weeks with the CER cream significantly reduced dryness/scaling/itchiness which was accompanied by significant decreases in TEWL and increases in capacitance values, at 2 and 4 weeks. In contrast, treatment for 4 weeks with the HIRU cream elicited a similar but lesser reduction in dryness/scaling/itchiness which was accompanied by significant but lesser decreases and increases in TEWL and capacitance values, respectively, at 2 and 4 weeks. Comparison of TEWL and capacitance values during the 4 weeks of treatment with either cream revealed that whereas the two parameters of CER cream treated skin were generally similar to healthy control skin, those of the HIRU cream treated skin remained similar to the mild or moderate AD skin. It is likely that the recovery in barrier function also reflects the improvement in clinically evaluated dry skin conditions, which suggests that the barrier replenishing effect is a more important factor for treatment of AD non-lesional skin than is the improvement of water deficiency.

P8. Skin Barrier Damage: Cause or Consequence of Atopic Dermatitis?

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The barrier to the penetration of irritants and allergens into the skin is located in the stratum corneum; it prevents the loss of water from the host, thereby maintaining internal homeostasis. The thickness of the stratum corneum varies considerably and is thinnest on the eyelids and flexural skin sites. Irritants and allergens may interact with sites of predisposition to atopic dermatitis (AD) that have a low stratum corneum barrier reserve and are therefore more prone to break down. Corneodesmosomes lock corneocytes together and prevent them from being dislodged by shearing forces. Corneocytes are shed from the skin surface by proteolysis, which is mediated by skin-specific proteases, such as stratum corneum chymotryptic enzyme (SCCE). These proteases are inhibited by skin-specific protease inhibitors, such as secretory leukocyte protease inhibitor (SLPI). It is essential that premature desquamation and breakdown of the skin barrier is prevented, a process that can permit the penetration of irritants and allergens, leading to the development of AD flares.

Recent increased interest in the role of the skin barrier in AD was initially triggered by the observation that a large proportion of children with AD (30–66%) did not have a raised non-specific or specific IgE. If these children were not immunologically atopic, how had the clinical AD developed? We investigated whether part of the genetic basis of AD could be due to changes in skin-barrier-related genes, such as SCCE. We screened the SCCE gene for variations and found an AACC insertion in the 3'UTR and then performed a case–control study, comparing patients with AD with unaffected controls; this demonstrated a strong genetic association between the AACC insertion and AD. This insertion may result in increased mRNA stability and increased SCCE protease levels leading to premature breakdown and thinning of the epidermal barrier. This allows the penetration of irritants and allergens, and triggers the development of eczematous lesions.

In AD, multiple environmental factors interact with changes in many genes to produce the disease phenotype. The application of some topical dermatological products can impair the epidermal barrier, e.g. certain emollients and prolonged use of potent topical corticosteroids. We used the tape stripping/TEWL method developed by Kao *et al* (2003)[6] to assess epidermal barrier function after the application of topical corticosteroids (TCs). The very potent TC clobetasol proprionate caused a major deterioration in skin barrier function after applying twice-daily for 5 days to normal human volunteer skin. The application of the potent TC betamethasone valerate 0.1% also damaged the skin barrier, but it took longer (15 days).

There is increasing evidence that one of the primary genetic defects in AD is in skin-barrier-related genes such as SCCE. This makes the skin barrier vulnerable to the penetration of irritants and allergens, which trigger AD flares. Environmental factors such as soap, detergents, exogenous proteases and very potent TCs may interact with the defective barrier in a genetically predisposed individual to unmask/exacerbate AD. Understanding the detrimental effects of TCs on the skin barrier, helps us learn how best to use TCs. In a severe flare the overall effect of a TC on the skin barrier is positive whereas, before and after a flare, the effect can be negative and is more likely to exacerbate than improve AD.

P9. Epidermal Abnormalities Underlying Defective Barrier in Netherton Syndrome

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Netherton syndrome (NS) is a rare autosomal recessive skin disorder characterized by ichthyosiform erythroderma, hair shaft defect and atopic manifestations with elevated serum levels of IgE. *SPINK5* is the defective gene in NS and encodes the multi-domain serine protease inhibitor LEKTI, strongly expressed in the granular layer of normal epidermis. The absence of LEKTI expression in the skin is a constant feature of NS. Significant associations also exist between polymorphisms in *SPINK5* and atopy and/or atopic dermatitis. We have recently characterized a mouse model for NS and demonstrated that unregulated epidermal protease activities result in the degradation of desmosomal proteins and the dysfunction of epidermal barrier. To address whether the degradation of desmosomal cadherins by unregulated proteases occurred in the epidermis of individuals with NS, we have analyzed the expression of desmosomal proteins and terminal differentiation markers by immunohistochemistry in the skin of 12 NS patients. Desmoglein-1 was present in the upper spinous layers but was remarkably reduced in the most differentiated nucleated layers of the epidermis in 7 patients. Desmocollin-1 and desmoplakin staining was also diminished in 4 patients in these layers. Corneodesmosin expression extended to the upper spinous layer in 3 patients. Involucrin and loricrin expression was enhanced and was redistributed in the most superficial spinous layers in the majority of cases analyzed. The anomalies of desmosomal proteins expression were associated with altered desmosome ultrastructure in the upper living layers of epidermis. Interestingly, there was a redistribution of SCTE immunostaining in 3 NS patients, in the same cellular layers where the expression of desmosomal components was reduced. *Stratum corneum* protease activities were increased in NS epidermis in comparison with controls as shown by *in situ* zymography. These results show that LEKTI plays a key role in the control of *stratum corneum* protease activities to maintain normal epidermal integrity and to form a functional permeability barrier.

Session 8: Evidence-Based Therapy, Education and Quality of Life

KL10. Updating The NHS HTA Systematic Review on Evidence-Based Treatments of Atopic Dermatitis

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The UK National Health Service systematic review of treatments for atopic dermatitis provided an important milestone in documenting our collective knowledge and areas of ignorance with regards to the treatment of this important disease. That review was published in the year 2000, which means that it is already out of date. Although the field of new randomised controlled trials in atopic dermatitis is moving at a modest rate, it is important to keep such systematic reviews updated in order for them to be clinically useful. In this talk, I shall present an update of the 80 or so randomised controlled trials on interventions for the prevention and treatment of atopic dermatitis that have published over the last 5 years since the HTA report was published. Since it is impossible to discuss every trial in a short talk, I shall concentrate on discussing some key developments including (i) probiotics (ii) topical calcineurin inhibitors (iii) ''weekend'' therapy for topical corticosteroids (iv) the ETAC study and (v) the evening primrose oil story. The update of the systematic review of atopic dermatitis treatments will be published in the public domain in November 2005 at www.nottingham.ac.uk/dermatology.

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IC9. The German Multicenter Trial of Education in Atopic Dermatitis

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Educational programmes aim to empower patients and/or carers in solving the problems arising from chronic diseases, and meta-analysis of results has highlighted the need to develop standardized methodologies so that any improvements in disease self-management can be more accurately assessed. Although several educational interventions have been developed for adult AD patients, the literature on educational programmes for children and their parents is sparse. The German Atopic Intervention Study (GADIS) was set up to develop standardized interventions for AD self-management, and to address their effects.

Standardized AD group intervention programmes were developed to educate parents of AD children 3 months to 7 years of age (Group 1); parents and their AD children aged 8–12 years (Group 2); and AD adolescents aged 13–18 years (Group 3). All patients had a confirmed diagnosis of AD, and a severity of eczema of at least 20 points on the SCORAD scale. After randomization to intervention (Group 1, $n = 274$; Group 2, $n = 102$; Group 3, $n = 70$) or to no education (Group 1, $n = 244$; Group 2, $n = 83$; Group 3, $n = 50$), parents and/or children in the intervention groups took part in six, weekly group sessions of 2 hours each. Efficacy was evaluated using the severity of eczema on the SCORAD scale, and standardized questionnaires for subjective severity. In programmes for the management of AD in children under 13 years of age, parental quality of life (QoL) was also assessed. The changes in the investigated parameters at the beginning of the study (T0) and 12 months after the end of the education programme (T1) were analysed using analyses of covariance. In all age groups, significant improvements in SCORAD severity and subjective severity of AD were seen in the intervention groups, compared with the control groups. Parents of AD children under 7 years of age experienced significantly better improvement in all five QoL subscales, while parents of AD children aged 8–12 years experienced significantly better improvement in three of five QoL subscales. It can be concluded that these educational programmes for the parental management of AD in children, and self-management of adolescents, improve disease control and should be integrated into routine care.

OC29. Parental Education in the Long-Term Management in Childhood Atopic Dermatitis

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Atopic dermatitis (AD) in childhood is a common inflammatory skin disease with the prevalence rates increasing. Its chronic course with frequent relapses puts a special burden on both children and their family. Moreover, in Korea, it is usual that the parents with AD patients have a low prudential to dermatologic remedy, because they traditionally believe that taking dermatologic medicine may be toxic to the body. Therefore, in order to maximize positive long-term outcome in the management of AD, it is important to support parents in dealing with the chronic condition of their child in addition to treating symptoms. The aim of this study was to determine the effect of a parental training program on managing AD in children. Two-hundred fifty seven families participated in this study. They attended weekly on the 4 educational programs for one month. The subject of each week composed of 1) the definition and the causes of AD, 2) the medical treatment for AD, 3) the environmental controls including skin care, and 4) a network therapy for AD. The families were assessed at the beginning of the study and 6 months later. Main outcome measures: severity of eczema (SCORAD); treatment behavior; quality of life; and coping strategies. Significant effects were shown regarding regular bathing and use of moisturizer, use of topical steroids in the event of exacerbation, and a significant reduction in the use of alternative therapies. Conclusively speaking, educational programs for parents of children with AD are a helpful adjunct to dermatological treatment.

OC30. An Audit of the Impact of a Consultation with a Paediatric Dermatology Team on Quality of Life in Infants with Atopic Eczema and Their Families: Further Validation of the Infants' Dermatitis Quality of Life Index and Dermatitis Family Impact Score

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Atopic dermatitis [AD] accounts for 10-20% of referrals to secondary care dermatology, often requiring multiple visits and occupying much valuable time and resources. We audited the usefulness of two simple and easy to use quality of Life (QoL) measures, the Infants' Dermatitis Quality of Life index (IDQOL) and Dermatitis Family Impact (DFI), in a routine clinical setting. The aims were to assess the impact on QoL of AD in infants and their families and the effect of an initial consultation with a paediatric dermatology team on AD severity and QoL impairment from the parents' perspective. The parents of 203 infants (mean age 19.8 months) with AD attending paediatric dermatology clinics completed the DFI and IDQOL. The parents of 50 of these infants completed both questionnaires before first and second consultations.

In the 203 children the mean of both the IDQOL and DFI score was 8.47 (median 8 and 7 and SD 5.8 and 6.5, respectively). The IDQOL and DFI correlated well ($rs = 0.79$, 95% CI 0.73-0.84). The parent's assessment of the global severity of AD correlated well with the IDQOL score ($rs = 0.60$, CI 0.5-0.69) but less well with the DFI ($rs = 0.4$, CI 0.27-0.51). The highest scoring IDQOL items were itching and scratching, problems at bath-time and time taken to fall asleep. The highest scoring DFI items were tiredness and exhaustion, sleep and emotional distress. In both measures these domains also correlated most strongly with eczema severity. After dermatology consultation the median global severity score rated by 50 parents, fell from 2 (SD 0.83) to 1 (SD 0.8) (CI 0.5-1), the median IDQOL score fell from 8 (SD 5.92) to 5 (SD 5.92) (CI 2-5.5) and the median DFI score fell from 9.62 (SD 6.45) to 5.49 (SD 6.56) (CI 2-5.5). In 50 infants the mean IDQOL scores for those infants with global AD severity scores of 1, 2 and 3 were 5 (SD 5.65), 8.1 (SD 4.27) and 12.9 (SD 5.67) respectively and improved by 28%, 24% and 49% respectively while the mean DFI scores by 40%, 35% and 50% respectively. The most improved IDQOL items were the time taken to get off to sleep and difficulty at mealtimes and the most improved DFI domains were tiredness and exhaustion and emotional distress in the parents.

We have provided further important information on the effects of AD on infants and their families using the IDQOL and DFI QoL measures. We demonstrate the usefulness of these measures in routine clinical management of AD and show the beneficial effect for both infants and parents of the initial consultation by a dermatology team in a secondary care setting.

OC31. Comparative Trial of Topical Corticosteroids in Atopic Dermatitis

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Background: Application of topical corticosteroid is a basic treatment in the management of atopic dermatitis. In Japan, topical corticosteroids are separated into five classes based on potency. Class 1 includes the most potent, while class 5 includes the least potent and Japanese dermatologists are recommended that they should use topical corticosteroids with appropriate potency according to the severity of the skin lesions. However, it is not clear that topical corticosteroids with the same potency are equally effective in ameliorating skin lesions of atopic dermatitis. Moreover, it remains to be determined whether clinical effectiveness of topical corticosteroid closely parallels the potency of the agent. In the present study, therefore, we performed comparative trial of topical corticosteroids in patients with atopic dermatitis.
Methods: A total of 1424 patients with atopic dermatitis were included in the study. Patients were instructed to apply one topical corticosteroid on the right side of the trunk and another topical corticosteroid on the left side of the trunk. Potencies of two topical corticosteroids were equal in 1212 patients (class 1 in 28 patients, class 2 in 682 patients, class 3 in 480 patients, class 4 in 22 patients), while potencies of two topical corticosteroids were different in 212 patients (class 1 vs class 2 in 12 patients, class 2 vs class 3 in 122 patients, class 3 vs class 4 in 78 patients). A week later we checked their skin condition to compare the clinical effectiveness of one topical corticosteroid with that of another topical corticosteroid. The skin condition was assessed on a five-point scale: markedly improved (>50% improvement), moderately improved (25% to 50% improvement), slightly improved (<25% improvement), unchanged or worsened. When skin lesion of one side markedly improved and skin lesion of another side slightly improved, unchanged or worsened, the clinical effectiveness of 2 kinds of topical corticosteroids were judged to be different.
Results: The clinical effectiveness of 2 kinds of topical corticosteroids were different in 210 (15%) of the 1424 patients examined. Topical corticosteroids with the same potency showed different clinical effectiveness in 117 (10%) of the 1212 patients, while topical corticosteroids with different potencies revealed different clinical effectiveness in 93 (44%) of the 212 patients. The topical corticosteroids with less potency showed more remarkable improvement than that with more potency in 32 (34%) of the 93 patients.
Conclusion: This study suggests that comparative application of topical corticosteroids is a useful way to find appropriate agents in the management of atopic dermatitis.

P11. Methotrexate Treatment of Atopic Dermatitis

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Background: Atopic dermatitis (AD) is a chronic inflammatory skin disease mediated by allergen-specific, type 1 and 2 cytokine-producing T cells which are recruited and activated in lesional skin. The therapies available for moderate to severe AD include topical agents (corticosteroids, immunomodulators) and systemic drugs (phototherapy, cyclosporin). Methotrexate is an old systemic agent used at low dosage for the treatment of psoriasis, another T cell-mediated skin disorder. The side effects are well-known, infrequent and in most cases not severe. More importantly, recent studies showed that MTX does not have broad immunosuppressive properties and behaves more as an anti-inflammatory molecule at the low doses used in dermatology. Indeed, MTX selectively targets activated T cells and has no effect on naïve and memory T cells. Therefore, we postulated that MTX could be a valuable and safe treatment of AD.
Study design: In the present open retrospective study, we show that low-dose MTX is an effective treatment of adult AD. Twenty patients (17 to 68 years old) with mild to severe AD and unresponsive to routine therapies were treated (three months to 2 years) with a weekly dose of MTX ranging from 7.5 to 25 mg, i.e. using the same protocol as that used in psoriasis. Laboratory tests were performed before treatment and every two weeks during the first three months and monthly thereafter (complete blood count, serum creatinine, aspartate aminotransferase, alanine aminotransferase).
Results: 75% of patients improved after 3 months of MTX use. The beginning of improvement was observed between the fourth and the sixth week after MTX was initiated. At 3 months, the improvement, based on physician global assessment and patient's evaluation of pruritus and quality of life, was >70% in 15/20 patients. MTX was stopped for three reasons: a high cumulative dose >1.5 g, dramatic improvement of AD and opportunity to start another treatment e.g. tacrolimus. Adverse events were nausea, hepatitis cytolysis with mild intensity. Side effects affected 6 patients and required discontinuation of MTX for 3 patients.
Discussion: From this open study, MTX could be considered as an effective and safe treatment of AD. Our study confirms the few previous reports of the efficacy of MTX in eczema (Egan et al.; Shaffrali et al). Controlled studies comparing MTX and placebo and MTX and cyclosporine are ongoing.

P10. Atopic Dermatitis and Cancer Risk

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Background: Epidemiological studies have provided growing evidence of a link between atopy and cancer risk.
Objective: The aim of this paper is to review the evidence from case-control studies and cohort studies on a possible association between atopic dermatitis (AD) and cancer risk, with particular attention to the case-definition of AD.
Methods: Studies with quantitative data on the association between AD (eczematous disease) and cancer risk were obtained from MEDLINE in combination with a review of cited references.
Results: In 23 publications, AD has been implicated in the risk of haematologic (childhood leukaemia (n = 3), adult leukaemia (n = 3), non-Hodgkin lymphoma (NHL) (n = 4) and different haematological cancers (n = 1)), pancreatic (n = 5), skin (n = 2) and brain malignancies (n = 5). The overall picture of the results of these studies shows that a history of AD may be associated with a decreased risk of pancreatic cancer, brain tumour and childhood leukaemia, although in most instances the findings were not statistically significant. No consistent associations were observed for skin cancer or Non-Hodgkin's Lymphoma. The definition of AD had varying quality, and was imprecise in many publications.
Conclusions: The findings of the epidemiological studies tend to support a lower risk of cancer among persons with a history of AD. Although a more careful definition of AD is needed, these epidemiological studies could provide an estimate of the background cancer risk in patients with AD when the long-term effects of treatments for AD are assessed (e.g., for topical immunomodulators).

Saturday 17 September 2005

Session 9: ETFAD Workshop on Allergy Testing in Atopic Dermatitis

0C32. Atopic Eczema and *Malassezia*

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The predominant allergic condition is atopic eczema (AE) with a prevalence of up to 20% among children and young adults in many parts of the world. Pathogenesis of AE is likely to result from defects in the immune system and a defective skin barrier, with both genetic and environmental factors contributing. Interestingly, microorganisms have been demonstrated not only to cause skin infections but also to trigger allergic symptoms by acting as allergens. One such microorganism is the yeast *Malassezia* formerly known as *Pityrosporum* which belongs to our normal cutaneous microflora but can cause skin infections and even systemic infections. *Malassezia* species are lipophilic yeasts commonly found on the body surface in humans and warm blooded animals. Several studies have shown that specific IgE and/or T-cell reactivity to *Malassezia* can be detected in a majority of adult patients with AE, and that antifungal treatment ameliorates the eczema and lowers the serum IgE levels (Scheynius A. *et al* Int Arch Allergy Immunol 2002;127: 161–9). We have therefore chosen *M. sympodialis*, the most common species isolated on skin both from healthy individuals and patients with AE, as a model for studies on host-microbe interactions to deepen our understanding of how a member of the normal skin flora can interact with the innate and adaptive immune system and contribute to the pathogenesis of AE. We have preliminary data indicating that the release of *M. sympodialis* allergens is significantly higher at pH 6, reflecting the higher pH in skin of patients with AE than that at pH 5 (normal skin pH). These data suggest that the skin barrier in AE patients provides an environment that can enhance the release of allergens from *M. sympodialis*, which can contribute to the perpetuation of the inflammation. So far we have successfully cloned, sequenced and characterized nine *M. sympodialis*-derived allergens, designated Mala s 1 and Mala s 5 - 12. Four of our identified *M. sympodialis* allergens are without homology to known proteins, while the others have potential cross-reactivity to human homologues like stress-induced heat shock proteins (HSP) and manganese super-oxide dismutase (MnSOD). We now have a unique opportunity to dissect pathogenic mechanisms in AE in relation to cross-reactivity with endogenous structures with the hypothesis that by molecular mimicry environmental fungal allergens might induce autoimmune reactivity to endogenous proteins. Obtained results will help clarifying whether *Malassezia* allergens can break immunologic tolerance possibly leading to an autoimmune response in AE. With our recombinant *M. sympodialis* allergens, we have created unique tools that have improved the diagnosis of AE using diagnostic tests like SPT and serology for detection of IgE-mediated reactivity. We have also developed an atopy patch test (APT) method and an *in vitro* lymphocyte proliferation assay to detect T cell-mediated reactivity against *M. sympodialis*. Interestingly, with our diagnostic tools we have found IgE-mediated sensitization against *M. sympodialis* in patients with the so called "intrinsic" form of AE, also denoted "non-atopic eczema". These patients present similar clinical symptoms but the underlying pathogenic mechanisms seem to differ. It is important to recognize the different subgroups of AE patients in order to identify underlying disease mechanisms and to offer the patients proper advice and treatment. We will now continue to improve the diagnostic methods to identify different subgroups of AE patients and to dissect underlying pathogenic mechanisms with the help of our unique recombinant allergens.

OC33. Studies with Aeroallergen Atopy Patch Tests

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Although IgE-mediated sensitizations to aeroallergens demonstrated by skin prick tests (SPT) or in vitro measurement of specific IgE-antibodies (sIgE) are very frequent in patients with atopic eczema (AE), these routine tests show a low specificity for clinically relevant allergy. For aeroallergens, more specific results can be obtained with the atopy patch test (APT).

In a European multicenter study on APT in 324 patients and controls using the ETFAD methods, positive aeroallergen APT reactions were seen in 10–39% (mostly to D. pteronyssinus) of patients, none in controls. Positive SPT (44–57%) and elevated sIgE (46–59%) were more frequent. Depending on the allergen, 20–34% of patients had a predictive history. 7–17% of patients had a clear-cut positive APT without positive SPT or elevated sIgE. Two of these patients with a corresponding history of AE triggered by house dust contact and with a positive APT to D. pteronyssinus showed sIgG4 against Der p 3. With regard to an aeroallergen-specific history, APT specificity ranged from 64–91%.

Currently, the APT is also used as inflammation model in trials on pathophysiology and topical and systemic therapy of AE. Such studies showed that APT reactions can be enhanced in volunteers after exposure to volatile organic compounds and can be modulated by pretreatment with pimecrolimus.

Aeroallergens can elicit eczematous skin lesions in patients with AE, also without corresponding sIgE. APT can be used to evaluate the clinical relevance with high specificity, the combination with SPT and sIgE is recommended.

OC34. Food Atopy Patch Test and Repeated Food Challenge

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Food hypersensitivity is a common medical problem in atopic dermatitis (AD) patients, mainly in paediatric age. Patch tests based on prolonged exposure of the skin to food, aiming at the detection of delayed reactions, represent a testing modality reproducing skin responses against allergens normally occurring in AD. Atopy patch tests (APTs) have been demonstrated to improve the accuracy of skin testing in the diagnosis of food allergy in AD patients; whereas immediate-type reactions proved to be associated with skin prick test positivity, APT reactivity is more frequently observed in patients with delayed responses. Relevance of positive and negative APT responses should be assessed by food challenge results.

Double-blind placebo-controlled food challenge (DBPCFC) is considered the gold standard for diagnosing food allergy, however, besides the fact that there is no universally accepted standard for performing the DBPCFC, these testing and observation modalities may not identify the whole spectrum of skin reactions possibly appearing in AD patients after food ingestion, which encompasses both prompt and eczematous responses sometimes appearing more than 48 hours after the challenge and requiring repeated administration of the foodstuff. Since many authors demonstrated that an open challenge associated to careful follow-up can prove adequate in identifying food allergic patients, also enabling the diagnosis of delayed reactions, the repeated open food challenge (ROFC) may be considered a useful and practical alternative to DBPCFCs.

In our experience this challenge modality seems to be well accepted and enables a good compliance. The subjects undergoing challenge are given a dose of the suspected food reflecting the normal intake daily at home for a week. The first administration is performed at the hospital in selected cases, depending on the nature and severity of the reaction to food allergens. When a clinical reaction is noted, the test is stopped and the patient is examined at the hospital. All subjects are examined on day 7 of the challenge.

APTs with egg, cow's milk and peanut were performed in 222 AD subjects undergoing repeated open challenges. 11%, 9% and 22% of them reacted to APT with egg, milk and peanut, respectively. Whereas sensitivity figures were low (31% for egg, 15% for cow's milk, and 37% for peanut), specificity was high (79% for egg, 92% for cow's milk, and 77% for peanut), indicating that a positive patch test enables the identification of food-allergic subjects.

OC35. SAFT and APT Using Fresh Foods in Children with Atopic Dermatitis and Food Allergy

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Atopic dermatitis [AD] is a chronic relapsing skin disease, usually beginning in infancy. AD is a multifactorial disease and several triggers have been recognized. Food allergy plays a role in a subgroup of infantile AD. A correct diagnosis of food allergy [FA] therefore is important to identify these children. Testing of FA is complicated and none of the current tests is completely decisive. In children with AD we perform allergological tests when they are suspected from food allergy. Most skin tests are based on the IgE-mediated type I hypersensitivity reaction. We perform the Skin Application Food Test (SAFT), according to Oranje, in children till the age of 3 years. This test is based on the mechanism of the 'contact urticaria syndrome' [CUS]. The food, in the way it is consumed, is put on the aluminum cup of a Finn Chamber on Scanpor tape. The test scores almost equal to an oral challenge. In children, older than 3 years, we prefer the prick-prick test. A small lancet is used to 'prick' in fresh food allergens, with a second 'prick' in the skin of the volar surface of the arm. Next to tests such as SAFT (skin application food test) and prick-prick test with fresh food, we also perform APT (atopy patch test) in SAFT-negative patients. Final proof will be obtained by the double-blind, placebo-controlled food challenge (DBPCOC), but in children younger than 2 years an open challenge is performed. The DBPCOC is considered the gold standard for diagnosing food allergy, but lacks exact definitions and therefore breakpoints. We found that the SAFT is equivalent to the oral challenge in children aged less than 3 years. The APT results only add a limited number of food allergic patients/cases.

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Devillers AC, Waard-van der Spek, de FB, Mulder PG, Oranje AP. Atopy Patch Test and SAFT as diagnostic tools in young children with atopic dermatitis and suspicion of food allergy. (in preparation)

OC36. Atopy Patch Test with Foods

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Atopy patch test (APT) has received much interest in recent years as a model for studying pathomechanism of atopic dermatitis (AD) and as a complementary test for diagnosing protein allergens causing or maintaining AD either by airborne contact or by food. From the time when the name of APT was given by Ring et al.in 1989 epicutaneous tests have been performed with allergens known to elicit IgE-mediated immediate reactions with the same allergen. Later a small subgroup of patients with AD has been identified to show positive APT with no positive circulating specific IgE to the same allergen. Even if the mechanism behind this reaction type is not totally clarified it opens new possibilities especially in the diagnosis of children with AE and food allergies but no specific IgEs.

The methodology of epicutaneous testing with aeroallergens and standardization of test materials has been extensively worked out in recent years by the group of Darsow and Ring (2000), but the standardization of APTs with foods still needs much work before it can be recommended for routine clinical diagnosis. In contrast to aeroallergens the sensitivity and specificity of APT in diagnose of food allergy can be calculated in relationship to challenge test with the same food. Unfortunately the challenge test itself is not exhaustively standardized.

For methodological aspects a 48 h occlusion time with readings at 48 h and 72 h seems convincing with foods, the skin of back is the best place for applying APT, and the APT seems to be fairly reproducible. There are no commercially available APT materials with foods so far. Well-planned multicenter studies are needed. The same is true for the reading of test reactions. Erythema with infiltration is regarded as minimal requirement for positive APT, but the role of the number of papules is not yet clear. In different papers different vehicles and control materials are used, some authors use tape stripping, others none. By the time being there are already more healthy controls tested with APT, but more studies in different age groups are needed. Adverse effects with APT are mild and include local flares with itching, contact urticaria, irritation from adhesive tapes. No active sensitisation has been reported with foods. There is no final agreement whether APTs with foods are equally practicable in different age groups.

There are several studies reporting sensitivity and specificity of APT for cow's milk, egg, and wheat. Even if the numbers vary the sensitivity for APT is mostly better than for skin prick test (SPT) but the specificities tend to be higher for SPT. The values need to be studied in multicenter studies with large patient material at the same age and with the same procedure for food challenge test.

At the time being food challenge test still remains the gold standard for diagnosing food allergy. The clinical picture of food allergy has changed and relatively simple cases with "only" milk and egg allergies have become rare, the modern picture of food allergy is multi-allergy. Multiple double blind placebo controlled food challenges cause stress to children and their parents as well as to the society in form of high costs. Therefore development of new additional tests is indicated for the accurate diagnose of food allergy and APT seems to be promising.

OC37. The Geneva Experience with Epicutaneous Tests 2000–2005 in Atopic Children

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Background: Corneotherapy is a baseline therapeutic approach in children with atopic dermatitis. Almost all available emollients contain preservatives, and many contain fragrances. Atopic dermatitis patients are characterized by a defective skin barrier and may thus be at a higher risk to develop contact sensitization to topical treatments.
Objective: To compare the frequencies of positive epicutaneous patch tests in atopic vs. non-atopic children.
Methods: Retrospective analysis of patch test results in children up to 15 years that were tested to both standard and preservatives series allergens since 2000 in two Geneva outpatient facilities. Analysis of patient files was completed by telephone interviews in the case of imprecise information about presence of atopic diathesis, i.e. past or present atopic dermatitis, atopic asthma, or rhinoconjunctivitis.
Results: A total of 56 children were identified that had presented one or several positive reactions to epicutaneous patch tests with both standard and preservative series. 43 children were atopic, 13 were non-atopic. 27/43 atopic patients were sensitized to one or several preservatives, as compared to only 1 of 13 non-atopic patients.
Conclusion: Children with atopic diathesis are more often sensitized to preservatives, than non-atopic children. This may reflect higher exposition because of intensive use of emollients, or because of the defective skin barrier. Prospective studies are needed in order to establish the causative pertinence of positive sensitizations to preservatives in atopic patients.

OC38. The Labial Food Challenge in Children with Atopic Eczema

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Atopic eczema is common in infants. In population based studies a cumulative prevalence of atopic dermatitis (AD) in childhood around 15-20% has been reported (1). Patients with atopic eczema have a strong systemic allergic response associated with marked elevations in serum IgE, activated eosinophils, and T cells. Food allergies play an important role in the pathogenesis of atopic eczema, affecting approximately one third of children with atopic eczema (1). Detection of these patients and identification of the offending foods will lead to marked improvement of the morbidity of AE.

Suspected food allergy should be confirmed using appropriate diagnostic tests (2). Skin Prick Testing and measurement of serum specific IgE will demonstrate sensitisation to the offending food. In case of delayed hypersensitivity atopy patch testing (APT) might be informative. Specific elimination diets implemented over a one to six week period may help identify trigger foods which are not immediately apparent. Examining children with atopic eczema, the "gold standard" in food allergy diagnosis is the double-blind placebo controlled food challenge (DBPCFC); however the procedure is time consuming, therefore expensive and poses a potential risk to the patient. Food challenges should only be performed by well-trained physicians in medical settings allowing management of anaphylactic reactions.

Labial food challenge (LFC) should be performed as the first step of an oral food challenge (3). It involves contact between the food and the labial mucosa, to test for local cutaneous manifestations. The anatomic characteristics of the lip make it suitable for allergy testing. The labial epithelium differs on different parts of the lip, and there is little keratinization outside the mouth and none inside and thus permeability is slight. The lip is red because of the rich vascularization of the chorion, which leads to even minor histamine type vasodilation causing visible edema. Finally, the labial mucosa contains mastocytes, which in the presence of even small amounts of allergen, are able to degranulate and release histamine and inflammatory mediators. This approach tends to avoid systemic reactions. The manifestations result from a local expression of the IgE response to the antigen. This technique can use both commercial extrats and crushed fresh food resuspended in physiological salt solution. A drop was placed on the lower lip and left for 2 minutes with the mouth slightly opened with a cotton swab between the lip and gum. The result was read 15 minutes later. Positive reactions in LFC can be divided into five types: 1) smoothing on the lower lip; 2) erythema under the lip; 3) edema of the cheek associated with rhinitis and watering eyes; and 5) systemic reaction associated with itching of the areas affected by eczema and coughing.

The LFC has several advantages. It is straightforward and takes less than 30 minutes. Only a single dose is required. Outpatients can be tested, at the same time as undergoing skin prick tests. The risk of systemic reactions is lower than that associated with the oral food challenge (3). The major drawback of the LFC is the difficulty in detecting weak reactions. The relatively poor sensitivity of the method requires oral food challenge after negative LFC results.

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P12. Is The Labial Food Challenge a Useful Tool in the Management of Food Allergy in Children with Atopic Dermatitis?

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The overall prevalence of food allergy in young children under 3 years is estimated between 4 −8%, and this prevalence being higher in atopic dermatitis (AD) children. The usual diagnosis of food allergy is made with skin prick testing and specific serum IgE assay followed by an oral food challenge (OFC). Recently, labial food challenge (LFC) was introduced as new diagnostic tool for food allergy (1). The objective of this study was to carry out a prospective comparison of OFC and LFC results regarding three common food allergens.
Patients and methods: We enrolled all the AD children with a suspected egg, peanut and/or cow's milk allergy referred to us for an OFC, from June 2001 to January 2005. After examination of the food allergy history, appropriate skin prick testing and specific IgE assays, LFC was performed immediately before OFC in a day hospital. Anti histamines were stopped at least 7 days before the tests. LFC were performed using a drop of commercial food extract of egg or peanut (*Stallergène, France*) or fresh milk put on the lower lip and left 10 seconds. The result was obtained 15 minutes later and positive reactions, mostly labial edema with or without contiguous urticaria, were graduated from 1 to 5 as previously described (1). OFC were then performed as single blinded tests using placebo and allergen extract capsules (*Lofarma*, Italy), followed by an open challenge with the concerned food, i.e. one egg, 3 peanuts or 150 ml of milk.
Results: An overall of 426 AD children (248 boys, 158 girls, aged from 1 to 12, mean age 4.02 years) were enrolled. Children were referred for egg, peanuts or milk allergy respectively in 228, 147 and 51 cases. LFC was positive in 110 cases and OFC in 136 cases, manifesting as urticaria (94), gastro-intestinal manifestations (75), asthma (29) and flare-up of eczema (15). Compared to the results of OFC, considered as the standard reference, the positive predictive value of LFC was 0.55, and the negative predictive value was 0.77. No difference was found between the three groups of food allergens. LFC caused systemic reactions in 2 cases (0.4%).
Conclusion: LFC is a simple and rapid to perform test in outpatients AD children, and it has been progressively and widely used in French allergist offices since the paper published by Rancé & coll. (1). Nevertheless, its predictive values and sensitivity are poor in AD children. We thus consider that it is premature to use the LFC as a substitute of OFC, but further studies are needed to investigate LFC as a part of a score including the results of skin-prick test and/or specific IgE assays.
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P13. The Comparison of Atopy Patch Test with Skin Prick Test in Korean Patients with Atopic Dermatitis

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Atopic dermatitis (AD) in childhood is frequently associated with food allergy. Allergic phenomena to food may be a consequence of both IgE- and non-IgE-mediated reactions. IgE-mediated mechanism can be evaluated by the skin prick test (SPT), while non-IgE mediated allergic responses by the atopy patch test (APT). Our aim was to investigate APT and SPT results to food allergens in 85 Korean with AD, and to further evaluate their relevance by performing repeated open challenges with positive food allergens. Eighty five patients suffering from AD and suspected food-related clinical symptoms were investigated with APT and SPT. The candidate food allergens were 30 kinds of plant or animal food. Of 40 APTs, egg white (39%), baker yeast (31%), cow milk (27%), beef (27%), chicken (27%), soybean (23%) were positive in order. Out of below 5 years with AD, egg white, beef, soybean, and cow milk were frequent in order, while, among above 6 years, egg white, egg yolk, pork, and baker yeast in order. When the SPT was performed in 45 ADs, baker yeast (47%), cow milk (30%), soybean (20%), and egg white (17%) were positive in frequency. Cow milk, soybean, egg white, and garlic were main positive allergen among below 5 years of ADs, while baker yeast, tuna, and soybean were positive allergen in order among above 6 years of ADs. In a meantime, we are performing open food challenges with positive allergens to confirm the relevance with APT or SPT, and will further present the results at the ISAD meeting.

P14. Gene Expression Change of PBMC using IGEC (Immune Gene Expression Chip) by Milk Stimulation Test in Milk Allergy of Atopic Dermatitis

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There is no convicing test for the diagnosis of food allergy except oral food challege test. IGEC (Immune Gene Expression Chip) was developed for the analysis of Gene expression. Gene expression change of PBMC by in-vitro milk stimulation using IGEC. A total of 50 Atopic dermatitis patients with or without milk allergy participated in this study. PBMCs were separated and stimulated with crude milk protein extract. The expression of 20 genes of cytokine, cytokine receptor and the gene for the intracellular signal transduction were evaluated. Gene expressions of PBMC were changed by milk stimulation. Characteristically, the gene expressions of STAT 1, STAT4 and CD40 Ligand were decreased in atopic dermatitis patients with milk allergy as compared to those without milk allergy. Conclusively, gene expression profile representative for food allergy were analyzed using IGEC, the gene expression DNA Chip in this study.

P15. Follow-Up Study of 92 Infants with Multiple Food Allergies

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Ninety-two infants <1 year of age (mean 6.1 $+$/-2.4 months) with atopic dermatitis (AD) and/or gastro-intestinal (g-i) symptoms were examined for the first time at our Allergy Unit. Skin prick tests (SPT) with large number of foods were performed in all of them and atopy patch tests (APT) with cow's milk (CM), egg, soy and cereals in 82 infants. Challenge tests were performed with CM and wheat. Sixteen infants needed only minor elimination guidelines and were followed-up at the well-baby clinics whereas 76 children were followed-up until the age of two years at the University Hospital. The outcome of symptoms with elimination of foods causing symptoms was recorded retrospectively.

Thirty-three children showed positive and 31 children negative reaction to open CM challenge. Sensitivity of SPT to CM was 0.30 and specificity 0.87 whereas the respective numbers with APT were 0.61 and 0.57. Open wheat challenge was positive in 19 and negastive in 26 children. Sensitivity for SPT was 0.47 and specificity 0.80, the corresponding numbers for APT were 0.82 and 0.17. In other children and with other foods the challenges were done at home and the patients were followed regularly and the allowed foods were recorded at every visit on a special form. The follow-up results are given for egg, CM and wheat, but several other foods were eliminated at the same time based on home challenges and compensated by suitable foods to guarantee normal growth.

At the age of 1/2 year(s) 10/8 children avoided egg, 2/1 children CM, 8/5 egg and CM, 25/16 egg and wheat (includes elimination of rye, barley and often oat), and 29/11 children egg, CM and wheat, respectively. Thirty-nine/54 children were asymptomatic at the age of 1/2 year(s). Careful diagnosis and elimination of culprit foods seems to be an adequate therapy and it is well accepted by the families.

P16. Alternaria Alternata Patch Tests on a Study Population of 500 Atopic Dermatitis Patients

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Introduction: Literature data on clinical symptoms related to exposure to Alternaria Alternata mostly concern subjects with respiratory atopy, whereas its role in the pathogenesis of atopic dermatitis (AD) has been poorly investigated. The aim of this study was to investigate Alternaria Alternata patch test results and to correlate the results to clinical history and specific IgE.

Methods: 500 patients with atopic dermatitis (AD), 222 males and 278 females, underwent patch testing with Alternaria Alternata 2.4% in petrolatum, provided by Lofarma (Milano, Italy). Specific IgE to Alternaria Alternata were detected by prick tests (Stallergenes, France).

Results: 38 (7,6%) patients, 13 males and 25 females, proved positive to Alternaria Alternata patch test. Among them, 12 subjects were affected by rhinitis and/or asthma. The most frequently involved skin sites in AD patients sensitised to Alternaria Alternata were the face, the flexural areas of the limbs and the neck. Among patients undergoing skin testing, 64 (12,8%) reacted to Alternaria Alternata prick tests.

Discussion: The significance of positive patch test responses to Alternaria Alternata patch tests remains questionable. The possibility that molds may be involved in the development of skin lesions in patch test-positive AD patients, should be checked with specific provocation and elimination procedures, which is particularly difficult, owing to the multifactorial etiology of AD.

P17. Allergic Sensitization to Common Inhalant Allergens and the Association with Atopic Diseases: Results of a Population Based in Elderly

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Background: There is a lack of epidemiological studies of atopic dermatitis and allergies in the elderly.

Objectives: To determine the prevalence of allergic sensitisation to common inhalant allergens and describe the main socio-demographic determinants of allergic sensitisation in a large population based sample of elderly. In addition we investigated the association of allergic sensitisation with the prevalence of physician diagnosed atopic dermatitis, hay fever, and asthma in individuals older than 50 years.

Methods: 4696 participants aged 50 to 74 years were recruited in the State of Saarland, Germany. All subjects filled out a standardized questionnaire and reported whether a physician had diagnosed atopic dermatitis, hay fever, or asthma. The presence or absence of specific IgE to common inhalants, like mite, oak, ragweed, grass, dog, cat and alternaria, was determined in the serum samples of all participants.

Results: Overall, we found a prevalence of 21.2% for allergic sensitization in the study population (mean age 61.9 years (SD $=$ 6.6)). The prevalence was 24.4% in males and 18.7% in females, decreased with increasing age (p trend $<$ .0001). Urban living in adolescence, a longer duration of school education and having a family history of atopy were associated with an increased risk for allergic sensitization. The prevalence of self-reported atopic dermatitis, hay fever, and asthma was 4.1%, 28.7%, and 10.8% among those with positive IgE response and 3.7%, 2.7%, and 4.0% in those without an IgE response.

Conclusion: The prevalence of allergic sensitization to inhalants appeared lower in the elderly compared to studies among younger adults. Even in our age group the prevalence decreased with increasing age. Sensitization to inhalants was associated with a high prevalence of respiratory symptoms such as hay fever and asthma but not atopic dermatitis in the elderly.

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KL11. Treatment of Atopic Dermatitis from a Skin Barrier Perspective

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There is increasing awareness that breakdown of the skin barrier may be one of the primary events in the development of atopic dermatitis. A recent systematic review of IgE measurements in children with atopic dermatitis revealed that in community studies up to 66% of the children did not have a raised IgE (specific or non-specific) at the point of sampling.[1] These children with mild moderate intrinsic atopic dermatitis can be regarded as having a primary transitory form of the disease.[2] One suggestion has been that there could be a primary defect in the skin barrier in atopic dermatitis[3] and this could be part of the explanation for intrinsic 'atopic' dermatitis.

The barrier to the penetration of irritants and allergens into the skin is located in the stratum corneum. Corneodesmosomes lock the corneocytes together and prevent them being dislodged by shearing forces. Corneocytes are shed from the surface of the skin by proteolysis, which is mediated by skin specific proteases, such as stratum corneum chymotryptic enzyme (SCCE). These proteases are inhibited by skin-specific protease inhibitors, such as secretory leukocyte protease inhibitor (SLPI). It is essential that premature desquamation and a breakdown of the skin barrier is prevented. A breakdown/thinning of the stratum corneum permits the penetration of irritants and allergens, which in turn can lead to the development of flares of atopic dermatitis.

In children with intrinsic atopic dermatitis, a strong genetic association has been demonstrated with an AACC insertion in the 3'UTR of the SCCE gene.[4] The most likely consequence of this insertion is to increase the levels of SCCE protein in the skin, leading to enhanced breakdown of the skin barrier and allowing the penetration of irritants and allergens, leading to a flare of atopic dermatitis.[4] Within a severe flare of atopic dermatitis there are also high levels of secondary and exogenous proteases which further enhance breakdown of the skin barrier.

The skin protease SCCE exhibits a neutral pH optima.[5] A change from pH 7.5 to 5.5 reduces SCCE activity by 50%.[6] The normal pH of the skin is 5.5, but washing with soap or detergents has been shown to raise the pH to 7.5 or more. Therefore, soap and detergents can cause a reduction in the thickness of the stratum corneum and induce flares of atopic dermatitis. Some emollients contain surfactants which have been associated with a large number of cutaneous reactions. Topical corticosteroids can cause a thinning of the stratum corneum as a result of damage to corneodesmosomes.[7,8] This effect appears to be mediated, at least in part, by the induction of skin proteases such as SCCE.[7]

The new understanding of the central role of skin barrier dysfunction in atopic dermatitis provides both an opportunity to develop new products and to use existing products more effectively. One example is topical corticosteroids which in some circumstances may cause damage to the skin barrier, and in others they may help repair it by inhibiting the production of secondary proteases.

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OC39. What Causes Flares of Atopic Eczema?

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Background: Many factors are quoted as causing flares in atopic eczema but with minimal objective evidence to support their roles.
Objectives: We sought to systematically search, summarise and critically appraise the evidence to support the roles of individual "flare" factors in atopic eczema.
Methods: We searched Medline and Embase from 1966 until March 2005 to identify relevant articles for inclusion in our review. There were no language restrictions. Meta-analysis was not possible due to differences in study methodology and populations. A descriptive summary of studies is therefore presented.
Results: Our preliminary data has identified 56 papers directly relevant to this review. 44 of these studies were experimental in design; 11 double blind placebo controlled food challenges (DBPCFC), 1 blinded direct exposure study to house dust mite, 13 open oral challenges, 10 non-blinded exposure studies (chlorine, UV, skin applied food tests, wool), 9 atopy patch studies and 2 inhalation challenges. Direct exposure studies, including experimental exposure and atopy patch testing to aeroallergens, suggest that house dust mite may have a significant role. Two inhalation studies have demonstrated that inhalation of aeroallergens can trigger eczema in participants. There were 12 observational studies: 7 questionnaire studies, 3 cohort studies and 2 anecdotal reports. Two recent observational studies in particular are highlighted. One inferred an association between stress, damp and heat and disease flares. The other study has suggested that there may be winter and summer types of eczema, the former flaring in cold weather and the latter when pollen counts are high.
Conclusions/Implications for practice and research: The relationship between climate and atopic eczema is not yet entirely clear. No definite conclusion can be drawn regarding other putative "flare factors". One reason might be that atopic eczema is a complex disease with flares caused by interactions of several exposures in combination, as opposed to factors working in isolation. Combined prospective observational and experimental studies are required to clarify roles of these factors. Knowledge of environmental flare factors, such as temperature, foods and dust, is important as many are modifiable thereby offering a non-drug approach to treating atopic eczema.

OC40. Differences in Percutaneous Absorption in Normal and Atopic Dermatitis Skin in Relation to Molecular Weight

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Molecular weight plays a significant role in the percutaneous penetration of chemicals. Atopic dermatitis is a chronic inflammatory skin disease associated with cutaneous hyperactivity to environmental triggers and is characterized by dry skin and increased transepidermal water loss.

Percutaneous penetration of chemicals and drugs might be higher not only in involved but also in non-involved skin of atopic dermatitis patients.The objective of this study was to investigate the differences in percutaneous penetration of polyethylene glycol (PEGs) in subjects with normal skin barrier and subjects with the history of atopic dermatitis in relation to the molecular weight. Twenty healthy and twenty subjects with history of atopic dermatitis were exposed to water solution of PEGs in range of 150–590 Da for 6 hours on the volar forearm. After the end of exposure the stratum corneum was totally removed by means of tape stripping and the concentration of PEGs and proteins were determined in each strip. Using Fick's second law of diffusion the permeation parameters were determined.

The penetration of all PEGs into the stratum corneum was enhanced in non-involved skin of atopic dermatitis patients. This suggests that even the non-involved skin in atopic dermatitis patients has a compromised barrier function.

OC41. N-3/N-6 Polyunsaturated Fatty Acids in a Group of Patients with Recalcitrant Atopic Dermatitis and the Influence of Balanced Japanese Traditional Diet

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Background: There are allergic and non-allergic aspects in the significance of diet. Among non-allergic influence of diet, n-3/n-6 polyunsaturated fatty acid (PUFA) unbalance has been pointed out to be related to the increase of atopic diseases. In Japan, adult patients with severe atopic dermatitis (AD) have been increasing in numbers as traditional diet has replaced by modern confused diet, in which there are the problems of excess intake of n-6 PUFAs and lack of n-3 PUFAs. We reported successfully treated cases with recalcitrant AD using diet and Kampo, Japanese traditional medicine (JTM), in addition to conventional therapy (eCAM 2004:1: 49–62, 145–156) and the existence of various viewpoints on diet (Adv Exp Med Biol. 2004; 546: 281–96). To clarify one of factors in the meaning of diet, we examined serum n-3/n-6 PUFAs in comparison with clinical severity during treatment of AD as a pilot study.

Methods: Subjects were consenting outpatients with recalcitrant AD treated at our clinic. For the treatment, in addition to the standard therapy, diet instruction was performed. Meats, fat, oil mainly rich in n-6 PUFAs and sweets were recommended to reduce from the menus. Patients were instructed to ingest diets mainly consisting of traditional food rich in rice, vegetables, pulse and seafood rich in n-3 PUFAs as a part of JTM. Serum fatty acids in fasting sera collected before and during therapy were measured by capillary gas chromatography after methylation of the serum samples.

Results: Serum fatty acids were examined in 112 patients. As a whole, n-3/n-6 ratio before treatment was 0.16 which was lower than recommended one, 0.33 for Japanese. Clinical symptoms improved during treatment and n-3 PUFAs increased with mild rise of n-3/n-6 ratio to 0.19. In a comparison of severity and n-3/n-6 ratio, no correlation was observed for the total fatty acid, but in patients with an eicosapentaenoic (20:5 n-3) acid/arachidonic (20:4 n-6) acid ratio of less than 0.3 before therapy, who comprised 70% of all patients, the severity and n-3/n-6 ratio were inversely related. Regarding the improvement rate of the symptoms, they improved as n-3 lipids increased.

Case 1: A 41-year-old man with severe AD who had habitual confused diet showed clinical improvement after adding balanced diet and Kampo on the standard treatment. His serum n-3/n-6 ratio changed from 0.103 to 0.184 during 23 months accompanied with SCORAD score change from 66.2 to 15.1. The amount of topical steroids, tacrolimus ointment and Kampo remedies used in a month decreased during following 2 years. Case 2: A 28-year-old woman with recalcitrant AD accompanied with severe pruritus was advised to avoid sweet drinks and to take more balanced Japanese diet. Her symptoms improved after 4 months treatment and her serum n-3/n-6 ratio changed from 0.137 to 0.223 with SCORAD change from 60.3 to 3.8. She reported recurrence followed unbalanced diet and made efforts to keep balanced diet during next 11 months and the status with SCORAD score 0 continued over 6 months.

Discussion: Not all but in a group of patients with recalcitrant AD, excess intake of n-6 PUFAs and decrease in n-3 PUFAs ingestion might be an aggravating factor. Balanced traditional Japanese diet in addition to conventional treatment succeeded to improve their symptoms. These data showed one of the reasons that such a diet has been helpful in the treatment of recalcitrant AD from the viewpoint of long-term effects.

P18. Evaluation of the Corticosteroid-Sparing Effect of an Emollient Milk in a Large Population of Infants Affected by Atopic Dermatitis

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If emollients are considered as standard therapy in the management of atopic patients (1,2), their corticosteroid-sparing effect in Atopic Dermatitis (AD) has never been clearly demonstrated.

The aim of this open label, randomised, multicentric study was to evaluate the corticosteroid-sparing effect of an emollient milk containing Oat Rhealba® extracts in an homogenous population, of infants aged less than 1 year old suffering from Atopic Dermatitis (AD).

Children with mild to severe AD (SCORAD index $\geq$ 20 and $\leq$ 70) were randomised in two parallel groups: a group with the emollient milk applied twice daily, and a group receiving no emollient. The use of topical corticoids (class II & III desonide corticoids) was allowed in the event of inflammatory flare-ups in both groups. The primary end point was measurement of the amount of topical steroids used (grams) at 3 and 6 weeks. The secondary end point was clinical rating changes (SCORAD index).

162 children (84 in the group with the emollient milk, and 78 in the group without) were analysed in ITT. Mean age was 6 months. SCORAD index at baseline was 36 in both groups.

At 3 weeks, the amount of class II (moderate potency) topical corticosteroids was decreased above 5% (non significant) and the class III (high potency) topical corticosteroids was significantly decreased above 45% ($p < 0.05$) in the group with emollient in comparison to the group without emollient.

At 6 weeks, the amount of class II (moderate potency) topical corticosteroids was decreased above 7% (non significant) and the class III (high potency) topical corticosteroids was significantly decreased above 42% ($p < 0.05$) in the group with emollient in comparison to the group without emollient.

At 3 weeks and at 6 weeks, the SCORAD index was significantly improved ($p < 0.0001$) with no difference between the two groups. At 6 weeks, the SCORAD index was significantly decreased greater than 54% in both groups. The tolerance of the emollient milk containing Oat Rhealba® extracts was judged good to excellent in 94% of cases.

We demonstrated in an large homogenous population of 162 atopic infants, a significant reduction of class III (high potency) topical steroids consumption ($> 42\%$) after 3 weeks and 6 weeks of use of a specific emollient milk containing Oat Rhealba® extract. This emollient milk appears as a safe and corticosteroid-sparing auxiliary treatment in Atopic Dermatitis management.

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P19. Refractory Atopic Dermatitis Associated with Cobalamine Deficiency Treated with a Single B12 Injection

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Allergic contact dermatitis is an itchy skin condition caused by an allergic reaction to material in contact with the skin. It arises some hours after contact with the responsible material, and settles down over some days providing the skin is no longer in contact with it. Allergic contact dermatitis is distinct from irritant contact dermatitis, in which a similar skin condition is caused by excessive contact with irritants. Irritants include water, soaps, detergents, solvents, acids, alkalis, and friction. Irritant contact dermatitis may affect anyone, providing they have had enough exposure to the irritant, but those with atopic dermatitis are particularly sensitive. Most cases of hand dermatitis are due to contact with irritants. The dermatitis is generally confined to the site of contact with the allergen, although severe cases may extend outside the contact area or it may become generalised. Sometimes the allergen is transmitted from the fingers so unexpected sites can be affected (e.g., the eyelids and genitals). Dermatitis is unlikely to be due to a specific allergen if the area of skin most in contact with that allergen is unaffected. The affected skin may be red, swollen and blistered or dry and bumpy. Cobalt is a metal found naturally in soil, dust, and seawater. It is usually found in association with nickel. Cobalt and its salts have many uses. Reactions to contact with cobalt in an allergic individual include allergic contact dermatitis and irritant dermatitis. Vitamin B12 injections administered to allergic individuals may produce a red, tender and itchy area around the site of the injection. Oral ingestion of vitamin B12 is known to cause intractable hand eczema is some patients. "Nickle" which is the most common cause of allergic contact dermatitis, is not associated with worsening of dermatitis following administration of Vitamin B12 and this feature is in contrast with cobalt allergy. In this article, a 21 year girl with nickle dermatitis will be presented who was refractory to usual treatments held for contact dermatitis. Following diagnosis of macrocytic anemia and treatment with Vitamin B12 injections, significant improvement in signs and sympthoms occured with no further recurrence.

P20. Does Prolonged Topical Application of Tacrolimus in Children Result in Systemic Accumulation?

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Systemic exposure following topical application of tacrolimus is minimal and decreases with time as the skin condition improves. However most studies have evaluated tacrolimus concentration in blood following topical application for less than one month.

We have evaluated tacrolimus blood concentration in 34 children (aged 2–14 years, mean age 7 years) with moderate to severe atopic dermatitis (mean SCORAD index 61.9, range 32.3–87.1) after 3, 6 and 12 months of repeated application of Tacrolimus ointment 0.03%.

The tacrolimus blood concentration was assayed using EMIT (enzyme multiplied immune assay technique). The lower limit of quantification of this method was 0.25ng/L.

All blood samples assayed were below the lower limit of quantification (ie $<$ 0.25ng/L).

Our preliminary data seem to indicate that there is no systemic accumulation of tacrolimus after repeated application for long periods ($\geq$ 3months).